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(54) Title: DOSING REGIMEN FOR GEMCITABINE HCV THERAPY

(57) Abstract: A dosage regiment for the treatment of a Flaviviridae infection, including a hepatitis C viral infection, that includes administering gemcitabine (or its salt, prodrug or derivative, as described herein) in a dosage range of approximately 50 mg/m^2 per day for between one and seven days (e.g. 1, 2, 3, 4, 5, 6, or 7 days) followed ba cessation of therapy. Viral load is optionally monitored over time, and after cessation, viral rebound is monitored. Therapy is not resumed unless a significant viral load is again observed, and then therapy for 1-7 days and more preferred, 1, 2 or 3 days is repeated. This therapy can be continued indefinitely to monitor and maintain the health of the patient.

DOSING REGIMEN FOR GEMCITABINE HCV THERAPY

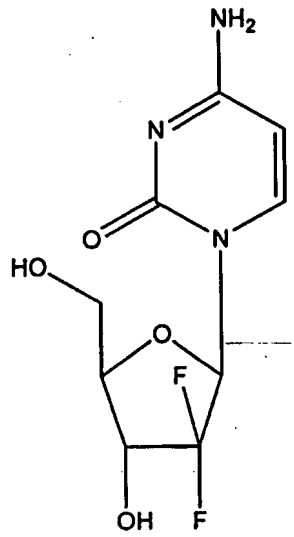
This application claims priority to U.S. patent application 60/357,411, filed on February 14, 2002, and U.S. patent application 60/358,140, filed on February 20, 2002.

FIELD OF THE INVENTION

The present invention is a method and dosing regimen for the treatment of a flavivirus or pestivirus, notably hepatitis C virus (HCV), using gemcitabine or its pharmaceutically acceptable salt or prodrug or a derivative thereof.

BACKGROUND OF THE INVENTION

Gemzar® (gemcitabine HCl) is a pyrimidine antimetabolite with antitumor activity against leukemias and a variety of solid tumors (e.g., pancreatic, non-small cell lung cancer, ovarian, breast, mesothelioma, etc.). Gemcitabine is a nucleoside analogue of the formula β-D-2',2'-difluorocytidine (see structure below). Gemcitabine was originally investigated for its antiviral effects but has since been developed as an antineoplastic agent. (Delong, D.C., L.W. Hertel, and J. Tang. Antiviral activity of 2',2' Difluorodeoxycytidine; *American Society of Microbiology*. 1986.) Gemcitabine has been approved by the Food and Drug Administration (FDA) for the following indications: (1) in combination with cisplatin as first-line treatment for patients with inoperable, locally advanced (Stage IIIA or IIIB) or metastatic (Stage IV) non-small cell lung cancer (NSCLC), (2) as a first-line treatment for patients with locally advanced (nonresectable Stage II or Stage III) or metastatic Stage (IV) adenocarcinoma of the pancreas and (3) as a second-line therapy for pancreatic cancer in patients previously treated with 5-fluorouracil (5-FU).



Gemcitabine

Gemcitabine (dFdC) is a cell cycle specific agent that primarily targets cells undergoing DNA synthesis (S-phase). Gemcitabine is metabolized intracellularly by the rate limiting enzyme deoxycytidine kinase (dCK) to its monophosphate form (dFdCMP). (Heinemann, V., et al., Comparison of the Cellular Pharmacokinetics and Toxicity of 2',2'-Difluorodeoxycytidine and 1-Beta-D-Arabinofuranosylcytosine. *Cancer Res.* 1988. 48(14): 4024-31). Subsequent phosphorylation by other nucleoside kinases leads to the formation of the active metabolites dFdCDP and dFdCTP. The cytotoxicity of gemcitabine is attributed to a combination of actions by the diphosphate and triphosphate metabolites that enhance the lethal effects of this agent. These actions are summarized in Fig. 1. First, dFdCDP inhibits ribonucleotide reductase (pathway 1) and this reduces the concentration of cellular deoxynucleotides (e.g., deoxycytidine triphosphate, dCTP) required for DNA replication (Figure 1; Self-Potentiating Actions of Gemcitabine and DNA repair). Reduced cellular dCTP concentrations that result from the inhibition of ribonucleotide reductase favor dFdCTP analog incorporation into DNA, an event critical for gemcitabine-induced lethality (pathway 2). (Huang, P. and W. Plunkett, Fludarabine- and Gemcitabine-Induced Apoptosis: Incorporation Of Analogs Into DNA Is A Critical Event. *Cancer Chemother Pharmacol*, 1995. 36(3):181-8; Huang, P. and W. Plunkett, Induction Of Apoptosis By Gemcitabine. *Semin Oncol*, 1995. 22(4 Suppl 11):19-25.). Reduced cellular dCTP levels also increase the rate of gemcitabine phosphorylation because high dCTP levels inhibit the rate limiting enzyme dCK (pathway 3). In contrast to its inhibitory effect on dCK, dCTP is a cofactor

required for the activity of dCMP deaminase, the rate-limiting enzyme for elimination of gemcitabine nucleotides from the cell (pathway 4). The cytotoxic metabolite dFdCTP directly inhibits dCMP deaminase (pathway 5). (Xu, Y.Z. and W. Plunkett, Modulation Of Deoxycytidylate Deaminase In Intact Human Leukemia Cells Action of 2',2'-difluorodeoxycytidine. *Biochem Pharmacol*, 1992. 44(9): 1819-27). And finally, at high intracellular concentrations FdCTP inhibits CTP synthetase (pathway 6) thereby blocking the synthesis of CTP, and consequently, that of dCTP as well. (Heinemann, V., et al., Gemcitabine: A Modulator Of Intracellular Nucleotide And Deoxynucleotide Metabolism. *Semin Oncol*, 1995. 22(4 Suppl 11):11-8).

Gemcitabine is a good substrate for phosphorylation by dCK to the monophosphate form dFdCMP, demonstrating a Km of 3.6 μmol/L with a substrate efficiency (Vmax/Km) similar to deoxycytidine (Km = 1.6 μmol/L as determined using a partially purified enzyme from Chinese Hamster ovary (CHO) cells. (Heinemann, V., et al., Comparison Of The Cellular Pharmacokinetics And Toxicity Of 2',2'- Difluorodeoxycytidine And 1-Beta-D-Arabinofuranosylcytosine. *Cancer Res*, 1988. 48(14): 4024-31). Phosphorylation of gemcitabine is essential for its biological activity and cells that lack dCK are not affected by gemcitabine. Studies with radioactive precursors of DNA, RNA and protein synthesis have demonstrated that the effects of gemcitabine are primarily directed at DNA. (Plunkett, W., et al., Gemcitabine: Preclinical Pharmacology And Mechanisms Of Action. *Semin Oncol*, 1996. 23(5 Suppl 10):3-15). Model systems of DNA synthesis confirmed that the triphosphate, dFdCTP, is incorporated into growing DNA primer strands by human DNA polymerases α and ε. (Huang, P., et al., Action Of 2',2'-Difluorodeoxycytidine On DNA Synthesis. *Cancer Res*, 1991. 51(22):6110-7). with each polymerase showing a 20-fold preference for the normal nucleotide (dCTP). Uniquely, incorporation of gemcitabine is followed by the addition of one more nucleotide before DNA polymerase is inhibited. When placed at the penultimate position, excision of dFdCMP by the 3' → 5' proofreading exonuclease proceeds at a much slower rate than excision of dCMP. This phenomenon described as "masked chain termination" improves the ability of gemcitabine to inhibit DNA replication and repair and provides a mechanism for synergism of gemcitabine with DNA damaging agents (e.g., cisplatin).

Gemcitabine is a good substrate for intracellular cytidine deaminase (Km = 96 μM), which is the enzyme responsible for the rapid metabolic clearance of gemcitabine via biotransformation to the deamination product 2',2'-difluorodeoxyuridine (dFdU) during clinical use. (Bouffard, D.Y., J. Laliberte, and R.L. Momparler, Kinetic Studies On 2',2'-

Difluorodeoxycytidine (Gemcitabine) With Purified Human Deoxycytidine Kinase And Cytidine Deaminase. *Biochem Pharmacol*, 1993. 45(9):1857-61). Gemcitabine is rapidly deaminated in the blood, liver, kidneys, and other tissues. Gemcitabine disposition was evaluated in 5 human subjects who received a single dose of radiolabeled drug 1000 mg/m^2 by 30 min infusion. Gemcitabine (<10%) and the inactive metabolite dFdU accounted for 99% of the excreted dose. The metabolite dFdU was also detected in the plasma and gemcitabine plasma protein binding was negligible.

The pharmacokinetics of gemcitabine was examined in 353 patients (2/3 men) with various solid tumors. Pharmacokinetic parameters were determined using data from patients treated for varying durations of therapy administered at weekly intervals with periodic rest weeks and using both short (< 70 min) and long infusions (70 to 285 min). The total gemcitabine dose administered ranged from 500 to 3600 mg/m^2. Gemcitabine pharmacokinetics are linear and described by the 2-compartment model. Elimination is dependent on renal excretion and clearance was influenced by age and gender. Population pharmacokinetic analyses of combined single and multiple dose studies determined that the volume of distribution of gemcitabine was significantly influenced by duration of infusion and gender. Gemcitabine half-life after short infusion ranged from 32 – 92 min and the value for long infusions varied from 245 to 638 min. These data reflected a greater volume of distribution with longer infusions. Volume of distribution was 50 L/m^2 following short infusions (< 70 min), indicating that gemcitabine is not extensively distributed in the tissues. Conversely, volume of distribution increased to 370 L/m^2 after long infusions, reflecting a slow equilibration of gemcitabine within the tissue compartment. The metabolite did not accumulate with weekly dosing but its elimination depends on renal excretion and dFdU levels may be influenced by renal impairment.

The effects of significant renal or hepatic insufficiency on gemcitabine disposition have not been assessed. The active metabolite dFdCTP can be extracted from peripheral blood mononuclear cells and the terminal phase half-life of dFdCTP from mononuclear cells ranges from 1.7 to 19.4 hours.

Importantly, the maximum tolerated dose (MTD) is heavily dependent on schedule and frequency of infusion. (Boven, E., et al., The Influence Of The Schedule And The Dose Of Gemcitabine On The Antitumour Efficacy In Experimental Human Cancer. *Br J Cancer*, 1993. 68(1):52-6). Prolongation of infusion time beyond 60 min and more frequent than weekly dosing has been shown to increase gemcitabine-related toxicity. Typically, myelosuppression is the dose-limiting toxicity manifested by leukopenia, thrombocytopenia,

and anemia. Patients should be monitored for myelosuppression during therapy because dosage adjustments for hematologic toxicity are frequently needed. Other toxicities associated with gemcitabine include stomatitis, nausea and vomiting, fever, rash, mild parasthesias, mild alopecia, flu-like symptoms (i.e., fever, chills, myalgia, cough, and headache) dypsnea, edema, mild proteinuria and hematuria, transient elevation of one or both serum transaminases, and diarrhea. Two clinical trials evaluated the efficacy of gemcitabine in patients with locally advanced or metastatic pancreatic cancer. The first trial compared gemcitabine with 5-FU in patients who had received no prior chemotherapy and the second trial evaluated patients who had received prior therapy with 5-FU or a 5-FU-containing regimen. In both studies gemcitabine was administered at a dose of 1000 mg/m^2 by 30 min infusion once weekly for 7 consecutive weeks (or until toxicity required withholding a dose) followed by one week of rest from treatment. Subsequent cycles consisted of weekly infusions for three consecutive weeks followed by one week of rest. The primary efficacy parameter in these studies was based on clinical benefit response defined and measured by improvements based on analgesic consumption, pain intensity, performance status and weight change. The first study was a multicenter, prospective, single blind, randomized comparison of gemcitabine and 5-FU in patients with locally advanced or metastatic pancreatic cancer. (Burris, H.A., 3rd, et al., Improvements In Survival And Clinical Benefit With Gemcitabine As First-Line Therapy For Patients With Advanced Pancreas Cancer: A Randomized Trial. *J Clin Oncol*, 1997. 15(6):2403-13). Gemcitabine was associated with statistically significant increases in clinical benefit response, survival, and time to disease progression compared to 5-FU with 63 patients evaluated in each treatment arm. The second trial was a multicenter open label study of gemcitabine in 63 patients previously treated with 5-FU or a 5-FU-containing regimen. (Rothenberg, M.L., et al., A Phase II Trial Of Gemcitabine In Patients With 5-FU-Refractory Pancreas Cancer. *Ann Oncol*, 1996. 7(4):347-53). The study showed a clinical benefit response rate of 27% with a median survival of 3.9 months.

Data from two randomized studies (657 patients) support the use of gemcitabine in combination with cisplatin for the first-line treatment of patients with locally advanced or metastatic NSCLC. One study compared gemcitabine plus cisplatin versus cisplatin alone and the second study evaluated gemcitabine plus cisplatin versus etoposide plus cisplatin. A total of 522 subjects were evaluated in the first study. (Mitchell, P.L., Quality Of Life And Cisplatin-Gemcitabine Chemotherapy. *J Clin Oncol*, 2000. 18(14): 2791-2). Gemcitabine (1000 mg/m^2) was administered on days 1, 8 and 15 of a 28-day cycle of cisplatin 100 mg/m^2 administered on day 1 of each cycle. Median survival time and median time to disease

progression were significantly greater in the gemcitabine plus cisplatin treatment arm compared to cisplatin alone. The objective response rate was 26% in the gemcitabine plus cisplatin treatment arm compared to 10% for cisplatin. In the second multicenter study 135 patients with stage IIIB or Stage IV NSCLC patients were treated with gemcitabine (1250 mg/m^2) on days 1 and 8 and cisplatin 100 mg/m2 on day 1 of a 21-day cycle or with etoposide 100 mg/m^2 I.V. on days 1, 2, and 3 and cisplatin 100 mg/m^2 on day 1 of a 21-day cycle. (Cardenal, F., et al., Randomized Phase III Study Of Gemcitabine-Cisplatin Versus Etoposide-Cisplatin In The Treatment Of Locally Advanced Or Metastatic Non-Small-Cell Lung Cancer. *J Clin Oncol*, 1999. 17(1):12-8). There was no significant difference in survival between the two treatment arms. Nevertheless, median time to disease progression and objective response rates were significantly greater in the gemcitabine plus cisplatin treatment arm compared to etoposide plus cisplatin.

Several cases of acute respiratory distress syndrome (ARDS) related to Gemcitabine treatment have been reported since 1997. These cases are associated with significant morbidity and mortality (Sabria-Trias et al. *Rev Mal Respir.* 2002. 19:645-7; Gupta et al. *Am J Clin Oncol.* 2002; 25(1):96-100). Gemcitabine pulmonary toxicity has been linked to the detection of Gemcitabine-induced systemic capillary leak syndrome (SCLS), a rare disorder with a high mortality rate, characterized by rapidly developing edema, weight gain and hypotension, hemoconcentration and hypoproteinemia, is caused by sudden, reversible capillary hyperpermeability with a rapid extravasation of plasma from the intravascular to the interstitial space. Recent evidence suggests that gemcitabine SCLS is the pathogenic mechanism for the pulmonary toxicity of gemcitabine (De Pas et al. *Ann Oncol.* 2001 12(11):1651-2). Further, it has been reported that the efficacy and safety of gemcitabine is more dependent on the schedule than on the dosage (Vermorken et al. *Br J Cancer* 1997 76(11):1489-93).

Although gemcitabine has been developed as an anticancer agent, there has been little serious investigation of gemcitabine as an antiviral agent for two reasons (1) those familiar with gemcitabine as an antitumor agent know that it is so toxic that it is usually be administered only according to a regimen of typically once a week for three to four weeks followed by a "rest week" (see Table 1 below); and (ii) standard antiviral therapy consists of daily administration of nucleoside analogues for an indefinite period, and perhaps for the life of the patient (see Table 2).

Table 1

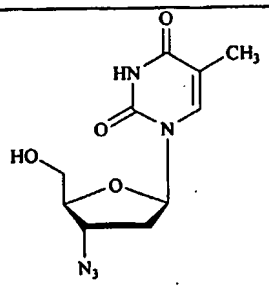
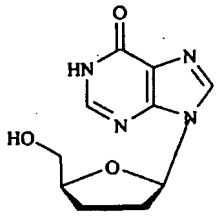
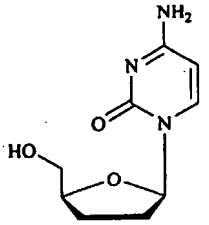
Standard Anticancer Dosages for Gemcitabine

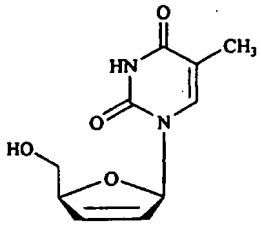
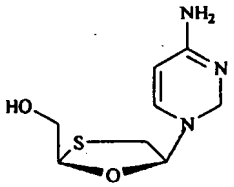
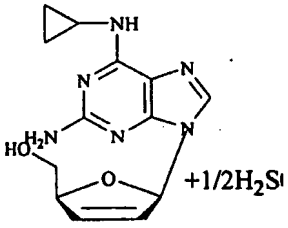
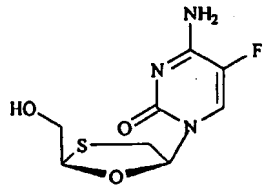
CANCER	INDICATIONS	DOSE REGIMEN	ADVERSE EFFECTS
Non-Small Cell Lung Cancer	For use in combination with cisplatin for the first-line treatment of patients with inoperable, locally advanced (Stage IIIA or IIIB) or metastatic (Stage IV) non-small cell lung cancer.	28 day cycle: gemcitabine (1250 mg/m^2, on days 1, 8 and 15) + cisplatin (100 mg/m^2 on day 1)	Thrombocytopenia, anemia, rash, vomiting, flu-like syndrome, fevers
Pancreatic Cancer	Treatment of patients with locally advanced (nonresectable stage II or III) or metastatic (stage IV) adenocarcinoma of the pancreas. Indicated for first-line treatment and for patients previously treated with a 5-fluorouracil-containing regimen.	1000 mg/m^2 over 30 minutes once weekly for up to 7 weeks	Thrombocytopenia, anemia, rash, vomiting, flu-like syndrome, fevers
Bladder Cancer		The recommended dose for gemcitabine is 800 - 1000 mg/m^2, given by 30 minute infusion. The dose should be given on Days 1, 8, and 15 followed by 1 week	Thrombocytopenia, anemia, rash, vomiting, flu-like syndrome, fevers

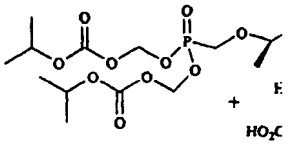
		rest. Optionally, Cisplatin is given at a dose of 70 mg/m^2 on Day 2 of each 28 day cycle.	

Table 2

Standard Antiviral Dosages for Nucleoside Analogues

Nucleoside Reverse Transcriptase Inhibitors						EC_{50} (μM)	
Formula and Name	Dosing*	Impact of monotherapy*	Adverse events*	Parent drug plasma-serum half-life (hr)*	NTP intracellular half-life (hr)	PBMC	T-cell lines
 **Zidovudine** [ZDV; AZT; azidothymidine; 1-(3'Azido-2'-deoxyribosyl) thymine; Retrovir®)	200 mg tid (6 pills per day) or 250 mg bid (2 pills per day)	One log decrease in HIV-1 RNA for six months to one year1	Nausea, vomiting, headache, neutropenia, anemia, insomnia	0.8-1.9	3-4	0.004-0.025	0.005-0.06
 **Didanosine** [ddI; 2',3'-dideoxyinosine; Videx®)	400 mg (twice daily for patients ≥60 kg) 250 mg (twice daily for patients <60kg)	~0.8 log decrease in HIV-1 RNA for six months to one year	Diarrhea, nausea, vomiting, peripheral neuropathy, pancreatitis	0.6-2.7	25-40	0.01	0.002-0.02
	0.75 mg tid (2 pills per day)	Less effective than either ddI or AZT	Peripheral neuropathy, mouth ulcers	1.0-3.0	2.6	0.02-0.16	0.03-0.05

Zalcitabine [ddC; 2',3'-dideoxycytidine; Hivid®)							
 **Stavudine** [d4T; 3'-deoxy-2',3'-didehycrothymidine; Zerit®)	40 mg (twice daily for patients ≥60 kg) 30 mg (twice daily for patients <60kg)	~0.8 log decrease in HIV-1 RNA for six months to one year	Peripheral neuropathy	1.0-1.6	3.5	0.009	0.001 -25
 **Lamivudine** [3TC; (-)-2',3'-dideoxy-3'-thiacytidine; Epivir®)	150 mg bid or 300 mg qd (approved October 2002)	Limited monotherapy data available	Nausea, headache, malaise, fatigue, diarrhea, cough	5.0-7.0	10.5-15.5	0.02-0.395	0.07-3.2
 **Abacavir** [ABC; TBC; Ziagen®)	300 mg bid, (2 pills per day)	Approximate 1.8 log reduction in HIV-1 RNA at four weeks	Nausea, vomiting, headache, (hypersensitivity reaction)	1.0-2.0	3.3	0.26-0.23	4.1
 **Emtricitabine** [FTC; Coviracil®)	NDA submitted	~2 log reduction at 14 days	Anemia	1.0-4.0	ND	0.0007-0.01	0.009 -0.5

 **Tenofovir disoproxil fumarate** [TDF; bis(POC)PMPA bix(isopropyloxymethylcarbonyl) 9-*R*-(2-phosphonomethoxypropyl) adenine, a prodrug of PMPA]	300 mg once daily (1 pill per day)	~1.2 log decrease in HIV-1 RNA (300 mg) at 28 days	Nausea, headache, asthenia, fatigue, diarrhea, vomiting, pharyngigtis, rash, cough, pain, rhinitis	17.0	10-50	0.03	0.04-8.5

A careful review of Table 2 indicates that antiviral therapy requires daily dosing over a long period of time to sustain a 1-2 log drop in viral load. It has been generally accepted by virologists that if therapy with antiviral drugs is stopped (or administered on an infrequent periodic basis) and virus has not been eliminated, the viral load will rebound quickly, and no sustained therapeutic effect will be achieved.

In 1986, Delong et al from Lilly Research Laboratories published the following abstract.

> Synthesis of a group of nucleosides containing 2',2'-Difluoro-2'-deoxyribose allowed us to examine their antiviral activity. Of particular interest was the cytidine analog which possessed very high in vitro activity against both RNA and DNA viruses without exhibiting toxicity in preformed monolayers. This compound also inhibited HSV-1 mutants resistant to FMAU and acycloguanosine that were thymidine kinase negative and with altered DNA polymerases. **Toxicity was observed in rapidly growing cells in culture.** The compound was tested in a variety of animal models for an antiviral effect. **Although the compound inhibited virus multiplication in acute virus infections in animals, we were unsuccessful in separating toxicity from virus activity**. However, we obtained very high activity in friend leukemia virus infections in mice that could be separated from toxicity by altering the dose schedule. Both spleen enlargement and polyerythroblastosis could be inhibited by 90% under conditions that allowed normal weight gain. A dose schedule calling for treatment every fifth day was possible. Activity was observed by both the oral and Ip routes. Studies were made which indicated

> that treatment could cause spleen size regression in mice that had enlarged spleens due to the infection.

Emphasis added. Abstracts of the Annual Meeting of the American Society for Microbiology (1986; Abstract No. T-56). While the authors did report an ability to separate activity from toxicity in the case of one viral infection (friend leukemia virus), this was apparently an isolated exception to the reported pattern of demonstrated inability to separate toxicity from activity.

U.S. Patent No. 5,015,743 discloses a genus of 2,2-difluoro-2-desoxycarbohydrate nucleosides, which includes gemcitabine, for the treatment of viral disorders. The patent teaches that "The antiviral nucleosides of the present invention are used for the treatment of viral infections in the manner usual in the treatment of such pathologies." In fact, it is now known that gemcitabine cannot be administered indefinitely on a daily basis in accordance with standard antiviral therapy. The patent includes one example of *in vitro* biological activity, "Test 1" in which the tested compound is not clearly identified. No in vivo data evaluating the toxicity was presented.

WO 02/18404 and US 2003/0008841 A1 filed by Hoffmann-La Roche, Inc. describe certain nucleoside derivatives for the treatment of hepatitis C. Gemcitabine is Compound 243 in Table 1 of the application, and Example 243. With regard to dosing, the Roche specification teaches that:

> The amount of the compound of formula I required for the treatment of hepatitis C virus infections will depend on a number of factors including the severity of the disease and the identity, sex and weight of the recipient and will ultimately be at the discretion of the attendant physician. In general, however, a suitable effective dose is in the range of 0.05 to 100 mg per kilogram of body weight of the recipient per day, preferably in the range 0.1 to 50 mg per kilogram of body weight per day and most preferably in the range of 0.5 to 20 mg of body weight per day. An optimum dose is about 2 to 16 mg per kilogram body weight per day. The desired dose is preferably presented as two, three, four, five, six or more sub-doses administered at appropriate intervals throughout the day. These sub-doses may be administered in unit dosage forms, for example, containing from 1 to 1500 mg, preferably from 5 to 1000 mg, most preferably from 10 to 700 mg of active ingredient per unit dosage form.

Again, the public is taught that it has to use these compounds, including gemcitabine on a daily basis, if not several times a day, to treat the viral infection. Because of the documented toxicity, this teaching at least with regard to gemcitabine appears to fall within the old adage

that "dead cells don't contain live virus." No reasonable physician, however, would kill or seriously damage a patient via chronic drug toxicity as a means to eliminate a viral infection. Therefore, regardless of these prior reports, no one has seriously considered the real world use of gemcitabine to treat a Flaviviridae infection, including HCV.

U.S. patent application no. 2002/0052317 and WO 02/10743 A1 disclose the use of erythropoietin to improve the tolerance to interferon, and which therapy may optionally also include the administration of one of a generic class of nucleoside analogs, including gemcitabine.

FlaviviridaeViruses, including Hepatitis C Virus

The *Flaviviridae* is a group of positive single-stranded RNA viruses with a genome size from 9-15 kb. They are enveloped viruses of approximately 40-50 nm. An overview of the *Flaviviridae* taxonomy is available from the International Committee for Taxonomy of Viruses. The *Flaviviridae* consists of three genera.

1. Flaviviruses. This genus includes the Dengue virus group (Dengue virus, Dengue virus type 1, Dengue virus type 2, Dengue virus type 3, Dengue virus type 4), the Japanese encephalitis virus group (Alfuy Virus, Japanese encephalitis virus, Kookaburra virus, Koutango virus, Kunjin virus, Murray Valley encephalitis virus, St. Louis encephalitis virus, Stratford virus, Usutu virus, West Nile Virus), the Modoc virus group, the Rio Bravo virus group (Apoi virus, Rio Brovo virus, Saboya virus), the Ntaya virus group, the Tick-Borne encephalitis group (tick born encephalitis virus), the Tyuleniy virus group, Uganda S virus group and the Yellow Fever virus group. Apart from these major groups, there are some additional Flaviviruses that are unclassified.

2. Pestiviruses. This genus includes Bovine Viral Diarrhea Virus-2 (BVDV-2), Pestivirus type 1 (including BVDV), Pestivirus type 2 (including Hog Cholera Virus) and Pestivirus type 3 (including Border Disease Virus).

3. Hepaciviruses. This genus contains only one species, the Hepatitis C virus (HCV), which is composed of many clades, types and subtypes.

HCV was not characterized until 1989 and had previously been referred to as non-A, non-B hepatitis. HCV, in combination with hepatitis B, accounts for 75% of all cases of liver disease worldwide. (Helbling, B., et al., Interferon And Amantadine In Naive Chronic Hepatitis C: A Doubleblind, Randomized, Placebo-Controlled Trial. *Hepatology*, 2002. 35(2):447-54). Liver failure related to HCV infection is the leading cause of liver transplants in the United States. Since HCV infection is typically mild in its early stages, it is rarely diagnosed until its chronic stages; therefore, HCV is often referred to as the "silent epidemic". The typical cycle of HCV from infection to symptomatic liver disease takes approximately 20 years, thus the true impact of this disease on the growing infected population will not be apparent for many years. HCV is spread by contact with the blood of an infected person. Individuals with the highest risk factors for HCV infection include:

- users of injectable illegal drugs
- recipients of blood transfusions or solid organ transplant recipients prior to 1992
- recipients of a blood product for clotting problems before 1987
- patients on long-term kidney dialysis
- individuals that exhibit evidence of liver disease (e.g., persistently abnormal ALT levels)

It is estimated that approximately 4 million people in the United States are infected with HCV, and more than 200 million persons are infected worldwide. (Hewitt, S.E., Recommendations for Prevention and Control of Hepatitis C Virus (HCV) Infection and HCV-related Disease. 1998, Centers for Disease Control and Prevention). During the 1980's an average of 230,000 new infections occurred each year. After 1989, the number of newly infected individuals declined by > 80% to 36,000 by 1986. Most of these persons are chronically infected and may be unaware of their infection because they remain asymptomatic. Thus, HCV-related liver disease is potentially one of the greatest public health threats to be faced in this century.

Chronic liver disease is the 10th leading cause of death among adults in the United States and accounts for 25,000 deaths annually. Population-based studies estimate that 40% of chronic liver disease is HCV-related. Since most HCV-infected persons are 30-49 years old, the number of deaths associated with HCV-related chronic liver disease may increase

substantially over the next 10 – 20 years. This is not trivial since current medical cost for treating HCV-related complications are estimated to be > 600 million dollars annually.

HCV is an RNA virus and this means that it mutates frequently. (www.epidemic.org/index2.html, The Facts about Hepatitis C. 1998, Dartmouth College). Once an infection occurs, HCV creates different genetic variations of itself within the body of the host. The mutated forms frequently differ from their precursors so the immune system cannot recognize them. Thus, even if the immune system succeeds against one variation, the mutant strains quickly take over and become predominate strains. This explains why >80% of individuals infected with HCV will progress to chronic liver disease. HCV has six major genotypes and more than 50 subtypes. In the United States among patients infected with HCV approximately 70% have genotype 1, 15% have genotype 2, and 10% have genotype 3. (McHutchison, J.G., et al., Interferon Alfa-2b Alone Or In Combination With Ribavirin As Initial Treatment For Chronic Hepatitis C. *Hepatitis Interventional Therapy Group. N Engl J Med*, 1998. 339(21):1485-92). Antiviral therapy is recommended for patients with chronic HCV infection who are at risk for progression to cirrhosis. (Herrine, S.K., *Approach To The Patient With Chronic Hepatitis C Virus Infection. Ann Intern Med*, 2002. 136(10):747-57). These persons include anti-HCV-antibody positive patients with persistently elevated ALT levels, detectable HCV RNA, and a liver biopsy that indicates either portal or bridging fibrosis or at least moderate inflammation or necrosis.

Therapy for HCV is rapidly changing and combination therapy with interferon and ribavirin, a nucleoside analog, is approved in the United States for treatment naïve patients with chronic HCV infection. (Hewitt, S.E., Recommendations for Prevention and Control of Hepatitis C Virus (HCV) Infection and HCV-related Disease. 1998, Centers for Disease Control and Prevention). Sustained response rates have been achieved in 40-50% of patients treated with ribavirin plus interferon compared to 15-25% with interferon alone. However, combination therapy in patients with genotype 1, the most prevalent HCV genotype in the United States, is not very successful and sustained response rates among these patients are still <30%. Furthermore, treatment-related side effects often lead to reductions in dose or discontinuation of treatment. Side effects frequently associated with interferon plus ribavirin therapy include, flu-like symptoms, irritability, depression, anemia, bone marrow suppression and renal failure. Ribavirin is teratogenic and contraindicated in women of child-bearing potential.

Due to the public health threat posed by chronic HCV infection and the limitations of current treatments, there is a growing need for innovative therapeutic approaches to treat HCV infection.

Therefore, an object of the present invention is to provide new compositions and methods for the treatment of *Flaviviridae*, and in particular HCV infection.

SUMMARY OF INVENTION

It has been surprisingly discovered that a minimal dose of gemcitabine (or its salt, prodrug or derivative, as described herein) can decrease the viral load of hepatitis C in a human patient by up to 2 logs or more in less than several days, and in fact, in certain cases, in 1-2 days or less. This observed rapid and large drop in viral load runs counter to conventional antiviral experience, wherein a drop of 1-2 logs is only stably achieved after approximately 14 days or more of daily sustained therapy. The unexpectedly robust and unique anti-HCV activity of gemcitabine or is salt or prodrug in a human provides the basis for a fundamental shift in the paradigm of antiviral drug dosing, and allows for the first time the conservative and appropriate use of the drug for such treatment.

Therefore, in a first embodiment, the invention provides a method and composition for the treatment of a Flaviviridae infection, and in particular, a hepatitis C viral infection, that includes administering gemcitabine (or its salt, prodrug or derivative, as described herein) in a dosage range of approximately 50 mg/m^2 to about 1300 mg/ m^2 per day for one, two or three days, followed by cessation of therapy. Viral load is then optionally monitored over time to evaluate viral rebound. Therapy is not resumed unless a significant viral load is again observed, and then therapy for 1, 2 or 3 days is repeated. This therapy can be continued indefinitely to monitor and maintain the health of the patient.

Flaviviridae viruses that can be treated include all members of the Hepacivirus genus (HCV), Pestivirus genus (BVDV, CSFV, BDV), and the Flavivirus genus (Dengue virus, Japanese encephalitis virus group (including West Nile Virus), and Yellow Fever virus).

In an alternative embodiment, for more severe Flaviviridae infections, gemcitabine (or its salt, prodrug or derivative, as described herein) is administered in a dosage range of approximately 50 mg/m^2 to about 1300 mg/ m^2 per day for between one and seven days (e.g.

1, 2, 3, 4, 5, 6, or 7 days) followed by cessation of therapy. Viral load is optionally monitored over time, and after cessation, viral rebound is monitored. Therapy is not resumed unless a significant viral load is again observed, and then therapy for 1-7 days (e.g., independently 1, 2, 3, 4, 5, 6 or 7 days) and more preferred, 1, 2 or 3 days, is repeated. This therapy can be continued indefinitely to monitor the and maintain the health of the patient.

For the first time, this invention discloses that antiviral therapy with gemcitabine or its salt or prodrug can be achieved using an anti-tumor dosing schedule. In certain embodiments, any approved anti-tumor dosage scheduling for gemcitabine can be used to treat a *Flaviviridae* infection.

In various illustrative and nonlimiting embodiments, the daily dosage of gemcitabine can range from 100-1500 mg per day, alternatively between 200-1000 mg per day, and more particularly between 300-800 mg per day.

In one illustrative embodiment, on Day 1, the patient is dosed via an intravenous infusion and then asked to remain at the clinic for several hours, up to perhaps 12 hours following administration of the dose of medication. The patient is monitored for safety and tolerance, and blood samples taken to measure HCV-RNA pre-dose, and then at 6 hours and 12 hours post-dose. On Day 2, the patient returns to the clinic for safety assessment and viral load measurements. Optional therapy is continued on days 2, 3, 4, 5, 6 and 7. Therapy is then ceased, and the patient is asked to return to the clinic periodically follow up safety and viral load testing.

It is preferred that gemcitabine be administered in the form of an intravenous infusion, because it is known that gemcitabine is rapidly converted to its uracil derivative in the digestive tract. If it is preferred to administer gemcitabine orally, then the compound should preferably be administered in the form of a prodrug that protects the cytosinyl amine group from rapid deamination without causing an adverse effect on activity. Nonlimiting methods to increase the half-life of the cytosine base in vivo include administering the compound in the N-acylated, N-alkylated or N-arylated form.

Prodrugs also include amino acid derivatives on either the hydroxyl or amino functions to create esters and amides of the disclosed nucleosides (*see, e.g.*, European Patent Specification No. 99493, which describes amino acid esters of acyclovir, specifically the glycine and alanine esters which show improved water-solubility compared with acyclovir itself, and US Pat. No. 4,957,924 (Beauchamp), which discloses the valine ester of acyclovir, characterized by side-chain branching adjacent to the α-carbon atom, which showed

improved bioavailability after oral administration compared with the alanine and glycine esters). A process for preparing such amino acid esters is disclosed in US Pat. No. 4,957,924 (Beauchamp). As an alternative to the use of valine itself, a functional equivalent of the amino acid may be used (*e.g.*, an acid halide such as the acid chloride, or an acid anhydride). In such a case, to avoid undesirable side-reactions, it may be is advantageous to use an amino-protected derivative.

As an example of the invention, a male patient exhibiting multifocal HCC, cirrhosis, and ischaemic hepatitis infected with HCV was administered 1200 mg gemcitabine HCl in 1000 minutes associated with oxaliplatine. The tolerance was acceptable, and thus the next day the patient was given a second dosage of approximately 700 mg of gemcitabine. Before the second dosage the baseline viral load was 6.49 log copies/mL. The second perfusion of gemcitabine was stopped after approximately 700 mg because of heart problems, which were apparently unrelated to the gemcitabine therapy. The HCV RNA measurement eight hours after the second dosage was 4.04 log copies/mL, indicating an approximate 2.5 log drop in eight hours.

In an alternative embodiment, gemcitabine or its salt, prodrug or derivative is administered according to the regimen described herein in combination or alternation with one or more other anti-Flaviviridae active agents. The other active agents (as described in more detail below) are administered in a manner that maximizes their effectiveness in combination with this regimen.

Brief Description of the Figures

Figure 1is an illustration of the self-potentiating actions of gemcitabine and DNA repair.

Figure 2 is a graphical depiction of the dose-dependant reduction of the replicon HCV RNA based on treatment with Gemcitabine (♦: ΔCt for HCV RNA). This viral reduction was compared to the reduction of cellular DNA levels (ribosomal DNA) or cellular RNA levels (ribosomal RNA) to obtain the therapeutic index ΔΔCt values (▲: HCV-rDNA ΔΔCt; **X:** HCV-rRNA ΔΔCt).

Detailed Description of the Invention

It has been surprisingly discovered that a minimal dose of gemcitabine can decrease the viral load of hepatitis C in a human patient by up to 2 logs or more in less than several days, and in fact, in certain cases, in 1-2 days or less. This observed rapid and large drop in viral load runs counter to conventional antiviral experience, wherein a drop of 1-2 logs is only stably achieved after approximately 14 days or more of daily sustained therapy. The unexpectedly robust and unique anti-HCV activity of gemcitabine in a human provides the basis for a fundamental shift in the paradigm of antiviral drug dosing, and allows for the first time the conservative and appropriate use of the drug for such treatment.

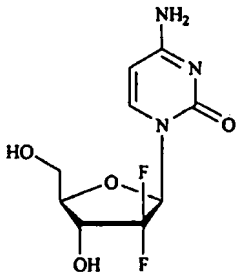
Therefore, in a first embodiment, the invention provides a method and composition for the treatment of a Flaviviridae infection, and in particular, a hepatitis C viral infection, that includes administering gemcitabine or its pharmaceutically acceptable salt or prodrug in a dosage range of approximately 50 mg/m^2 to about 1300 mg/ m^2 per day for one, two or three days, followed by cessation of therapy. Viral load is then optionally monitored over time to evaluate viral rebound. Therapy is not resumed unless a significant viral load is again observed, and then therapy for 1,2 or 3 days is repeated. This therapy can be continued indefinitely to monitor the and maintain the health of the patient.

Flaviviridae viruses that can be treated include all members of the Hepacivirus genus (HCV), Pestivirus genus (BVDV, CSFV, BDV), and the Flavivirus genus (Dengue virus, Japanese encephalitis virus group (including West Nile Virus), and Yellow Fever virus).

In an alternative embodiment, for more severe Flaviviridae infections, gemcitabine or its pharmaceutically acceptable salt or prodrug is administered in a dosage range of approximately 50 mg/m^2 to about 1300 mg/ m^2 per day for between one and seven days, followed by cessation of therapy. Viral load is then optionally monitored over time to evaluate viral rebound. Therapy is not resumed unless a significant viral load is again observed, and then therapy for 1-7 days, and more preferred, 1,2 or 3 days, is repeated. This therapy can be continued indefinitely to monitor and maintain the health of the patient.

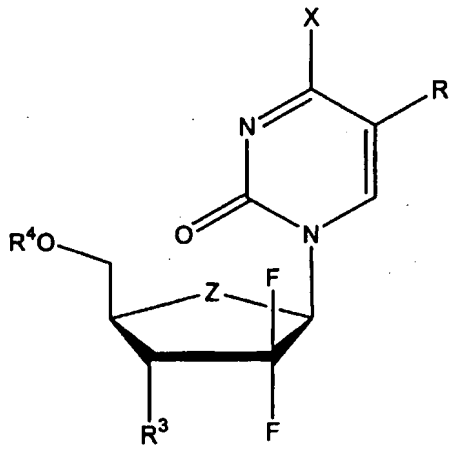
I. Compounds of the Invention

In a particular embodiment of the present invention, a β-D nucleoside of the formula:



its β-L-enantiomer, or its pharmaceutically acceptable salt or prodrug, is provided for the treatment or prophylaxis of a *Flaviviridae* infection, and in particular HCV. In a preferred embodiment, the compound is gemcitabine or its pharmaceutically acceptable salt, ester or prodrug. The compound, by way of example, can be alkylated, acylated, or otherwise derivatized at the N^4 and/or 3' and/or 5'-position to modify its activity, bioavailability, stability or otherwise alter the properties of the nucleoside. This may make it more stable for non-intravenous formulations. In one embodiment, the compound is acylated at the N^4 and/or 3' and/or 5' position with an amino acid, such as valine.

In a broader aspect of the invention, the active compound is a β-D or β-L nucleoside of the general formula (I): or its pharmaceutically acceptable salt or prodrug thereof (referred to herein as a gemcitabine derivative) wherein:



- R is H, halogen (F, Cl, Br, I), OH, OR', SH, SR', NH_2, NHR', NR'_2, lower alkyl of C_1 -C_6, halogenated (F, Cl, Br, I) lower alkyl of C_1-C_6 such as CF_3 and CH_2CH_2F, lower alkenyl of C_2-C_6 such as $CH=CH_2$, halogenated (F, Cl, Br, I) lower alkenyl of C_2-C_6 such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C_2-C_6 such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C_2-

C_6, lower alkoxy of C_1-C_6 such as CH_2OH and CH_2CH_2OH, CO_2H, CO_2R', $CONH_2$, $CONHR'$, $CONR'_2$, $CH=CHCO_2H$, $CH=CHCO_2R'$;

- X is H, halogen, OH, OR', OCH_3, SH, SR', SCH_3, NH_2, NHR', NR'_2, CH_3;
- each R' is independently a hydrogen, lower alkyl of C_1-C_6 or lower cycloalkyl of C_1-C_6;
- Z is O, S or CH_2;
- R^4 is H, mono-phosphate, di-phosphate, tri-phosphate; a stabilized phosphate prodrug; acyl; alkyl; sulfonate ester; a lipid, a phospholipid; an amino acid; a carbohydrate; a peptide; a cholesterol; or other pharmaceutically acceptable leaving group which when administered *in vivo* is capable of providing a compound wherein R^4 is H or phosphate; and
- R^3 is F or OH.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is halogen or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is alkyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is halogenated alkyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is CH_3 and R is NH_2 or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is OR' and R is halogen or its

pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2, R is halogen, R^4 is hydrogen or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is alkenyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is alkynl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is halogenated alkenyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is halogen alkynyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is alkoxy or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is CO_2H or its

pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is CO_2R' or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is $CONH_2$ or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is CONHR' or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R is halogen or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2, R^3 is OH, and R is halogen or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2, R^3 is OH, and R is alkyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2, R^3 is OH, and R is halogenated alkyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is CH_3 , R^3 is OH, and R is NH_2 or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is OR' , R^3 is OH, and R is halogen or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 , R^3 is OH, R is halogen, R^4 is hydrogen or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 and R^3 is OH, or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is CH_3 , R^3 is F, and R is alkenyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is CH_3 , R^3 is OH, and R is alkynl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 , R^3 is OH, and R is halogenated alkenyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2 , R^3 is OH, and R is halogen alkynyl

or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2, R^3 is OH, and R is alkoxy or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2, R^3 is OH, and R is CO_2H or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2, R^3 is OH, and R is CO_2R' or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2, R^3 is OH, and R is $CONH_2$ or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2, R^3 is OH, and R is CONHR' or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2, R^3 is OH, and R is halogen or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NH_2, R^3 is OH, and R is halogen, and R^4 is H or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is SH_2, R^3 is OH, and R is halogen or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NHR', R^3 is OH, and R is halogen or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R is halogen or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R is alkyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R is alkenyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R is alkynyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R is halogenated alkenyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R is halogenated alkynl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R is alkoxy or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R is CO_2H or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is OR' or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is NHR' or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein X is $CONH_2$ or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R^3 is F or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R^3 is OH or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R^4 is H or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R^4 is mono-phosphate or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R^4 is di-phosphate or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R^4 is tri-phosphate or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R^4 is acyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R^4 is H and Z is O or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R^4 is H and Z is CH_2 or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R is H, R^3 is F, and R^4 is acyl or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

In another embodiment of the present invention, the active compound is a β-D or β-L nucleoside of the general formula (I), wherein R^4 is H and R is OR' or its pharmaceutically acceptable salt or prodrug thereof or its use as further described herein is provided.

Definitions

The term "alkyl," as used herein, unless otherwise specified, refers to a saturated straight, branched, or cyclic, primary, secondary, or tertiary hydrocarbon, including but not limited to those of C_1 to C_{16}, and specifically includes methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl, *t*-butyl, pentyl, cyclopentyl, isopentyl, neopentyl, hexyl, isohexyl, cyclohexyl, cyclohexylmethyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl. The alkyl group can be optionally substituted with one or more moieties selected from the group consisting of alkyl, halo, haloalkyl, hydroxyl, carboxyl, acyl, acyloxy, amino, amido, carboxyl derivatives, alkylamino, dialkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, azido, thiol, imine, sulfonic acid, sulfate, sulfonyl, sulfanyl, sulfinyl, sulfamonyl, ester, carboxylic acid, amide, phosphonyl, phosphinyl, phosphoryl, phosphine, thioester, thioether, acid halide, anhydride, oxime, hydrozine, carbamate, phosphonic acid, phosphate, phosphonate, or any other viable functional group that does not inhibit the pharmacological activity of this compound, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, *et al.*, Protective Groups

in Organic Synthesis, John Wiley and Sons, Second Edition, 1991, hereby incorporated by reference.

The term "lower alkyl," as used herein, and unless otherwise specified, refers to a C_1 to C_4 saturated straight, branched, or if appropriate, a cyclic (for example, cyclopropyl) alkyl group, including both substituted and unsubstituted forms.

The term "alkylene" or "alkenyl" refers to a saturated hydrocarbyldiyl radical of straight or branched configuration, including but not limited to those that have from one to ten carbon atoms. Included within the scope of this term are methylene, 1,2-ethane-diyl, 1,1-ethane-diyl, 1,3-propane-diyl, 1,2-propane-diyl, 1,3-butane-diyl, 1,4-butane-diyl and the like. The alkylene group or other divalent moiety disclosed herein can be optionally substituted with one or more moieties selected from the group consisting of alkyl, halo, haloalkyl, hydroxyl, carboxyl, acyl, acyloxy, amino, amido, carboxyl derivatives, alkylamino, azido, dialkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, thiol, imine, sulfonyl, sulfanyl, sulfinyl, sulfamonyl, ester, carboxylic acid, amide, phosphonyl, phosphinyl, phosphoryl, phosphine, thioester, thioether, acid halide, anhydride, oxime, hydrozine, carbamate, phosphonic acid, phosphonate, or any other viable functional group that does not inhibit the pharmacological activity of this compound, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, *et al.*, Protective Groups in Organic Synthesis, John Wiley and Sons, Second Edition, 1991, hereby incorporated by reference.

The term "aryl," as used herein, and unless otherwise specified, refers to phenyl, biphenyl, or naphthyl, and preferably phenyl. The term includes both substituted and unsubstituted moieties. The aryl group can be substituted with one or more moieties selected from the group consisting of bromo, chloro, fluoro, iodo, hydroxyl, azido, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, *et al.*, Protective Groups in Organic Synthesis, John Wiley and Sons, Second Edition, 1991.

The term amino acid includes naturally occurring and synthetic α, β γ or δ amino acids, and includes but is not limited to, alanyl, valinyl, leucinyl, isoleuccinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycinyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaroyl, lysinyl, argininyl, histidinyl, β-alanyl, β-valinyl,

β-leucinyl, β-isoleuccinyl, β-prolinyl, β-phenylalaninyl, β-tryptophanyl, β-methioninyl, β-glycinyl, β-serinyl, β-threoninyl, β-cysteinyl, β-tyrosinyl, β-asparaginyl, β-glutaminyl, β-aspartoyl, β-glutaroyl, β-lysinyl, β-argininyl, and β-histidinyl.

The term "aralkyl," as used herein, and unless otherwise specified, refers to an aryl group as defined above linked to the molecule through an alkyl group as defined above. The term "alkaryl" or "alkylaryl" as used herein, and unless otherwise specified, refers to an alkyl group as defined above linked to the molecule through an aryl group as defined above. In each of these groups, the alkyl group can be optionally substituted as describe above and the aryl group can be optionally substituted with one or more moieties selected from the group consisting of alkyl, halo, haloalkyl, hydroxyl, carboxyl, acyl, acyloxy, amino, amido, azido, carboxyl derivatives, alkylamino, dialkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, thiol, imine, sulfonyl, sulfanyl, sulfinyl, sulfamonyl, ester, carboxylic acid, amide, phosphonyl, phosphinyl, phosphoryl, phosphine, thioester, thioether, acid halide, anhydride, oxime, hydrozine, carbamate, phosphonic acid, phosphonate, or any other viable functional group that does not inhibit the pharmacological activity of this compound, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, *et al.*, Protective Groups in Organic Synthesis, John Wiley and Sons, Second Edition, 1991, hereby incorporated by reference. Specifically included within the scope of the term aryl are phenyl; naphthyl; phenylmethyl; phenylethyl; 3,4,5-trihydroxyphenyl; 3,4,5-trimethoxyphenyl; 3,4,5-triethoxy-phenyl; 4-chlorophenyl; 4-methylphenyl; 3,5-di-tertiarybutyl- 4-hydroxyphenyl; 4-fluorophenyl; 4-chloro-1-naphthyl; 2-methyl-1-naphthylmethyl; 2-naphthylmethyl; 4-chlorophenylmethyl; 4-*t*butylphenyl; 4-*t*-butylphenylmethyl and the like.

The term "alkylamino" or "arylamino" refers to an amino group that has one or two alkyl or aryl substituents, respectively.

The term "halogen," as used herein, includes fluorine, chlorine, bromine and iodine.

The term "enantiomerically enriched" is used throughout the specification to describe a nucleoside which includes at least about 95%, preferably at least 96%, more preferably at least 97%, even more preferably, at least 98%, and even more preferably at least about 99% or more of a single enantiomer of that nucleoside. In a preferred embodiment, the nucleoside analog is provided in enantiomerically enriched form.

The term "host," as used herein, refers to a unicellular or multicellular organism in which the virus can replicate, including cell lines and animals, and preferably a human. Alternatively, the host can be carrying a part of the viral genome, whose replication or function can be altered by the compounds of the present invention. The term host specifically refers to infected cells, cells transfected with all or part of the viral genome and animals, in particular, primates (including chimpanzees) and humans. Relative to abnormal cellular proliferation, the term "host" refers to unicellular or multicellular organism in which abnormal cellular proliferation can be mimicked. The term host specifically refers to cells that abnormally proliferate, either from natural or unnatural causes (for example, from genetic mutation or genetic engineering, respectively), and animals, in particular, primates (including chimpanzees) and humans. In most animal applications of the present invention, the host is a human patient. Veterinary applications, in certain indications, however, are clearly anticipated by the present invention (such as bovine viral diarrhea virus in cattle, hog cholera virus in pigs, and border disease virus in sheep).

The term "pharmaceutically acceptable salt or prodrug" is used throughout the specification to describe any pharmaceutically acceptable form (such as an ester, phosphate ester, salt of an ester or a related group) of a compound which, upon administration to a patient, provides the active compound. Pharmaceutically acceptable salts include those derived from pharmaceutically acceptable inorganic or organic bases and acids. Suitable salts include those derived from alkali metals such as potassium and sodium, alkaline earth metals such as calcium and magnesium, among numerous other acids well known in the pharmaceutical art. Pharmaceutically acceptable prodrugs refer to a compound that is metabolized, for example hydrolyzed or oxidized, in the host to form the compound of the present invention. Typical examples of prodrugs include compounds that have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated, phosphorylated, dephosphorylated to produce the active compound.

The compounds of this invention either possess antiviral activity against *Flaviviridae* viruses or anti-proliferative activity against abnormal cellular proliferation, or are metabolized to a compound that exhibits such activity.

Stereoisomerism and Polymorphism

Compounds of the present invention have at least two chiral centers, and may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. The present invention encompasses racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein. The optically active forms can be prepared by, for example, resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase or by enzymatic resolution.

Optically active forms of the compounds can be prepared using any method known in the art, including by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chroma tographic separation using a chiral stationary phase.

Examples of methods to obtain optically active materials include at least the following.

i) physical separation of crystals - a technique whereby macroscopic crystals of the individual enantiomers are manually separated. This technique can be used if crystals of the separate enantiomers exist, i.e., the material is a conglomerate, and the crystals are visually distinct;

ii) simultaneous crystallization - a technique whereby the individual enantiomers are separately crystallized from a solution of the racemate, possible only if the latter is a conglomerate in the solid state;

iii) enzymatic resolutions - a technique whereby partial or complete separation of a racemate by virtue of differing rates of reaction for the enantiomers with an enzyme;

iv) enzymatic asymmetric synthesis - a synthetic technique whereby at least one step of the synthesis uses an enzymatic reaction to obtain an enantiomerically pure or enriched synthetic precursor of the desired enantiomer;

v) chemical asymmetric synthesis - a synthetic technique whereby the desired enantiomer is synthesized from an achiral precursor under conditions that produce asymmetry (i.e., chirality) in the product, which may be achieved using chiral catalysts or chiral auxiliaries;

vi) diastereomer separations - a technique whereby a racemic compound is reacted with an enantiomerically pure reagent (the chiral auxiliary) that converts the individual enantiomers to diastereomers. The resulting diastereomers are then separated by chromatography or crystallization by virtue of their now more distinct structural differences and the chiral auxiliary later removed to obtain the desired enantiomer;

vii) first- and second-order asymmetric transformations - a technique whereby diastereomers from the racemate equilibrate to yield a preponderance in solution of the diastereomer from the desired enantiomer or where preferential crystallization of the diastereomer from the desired enantiomer perturbs the equilibrium such that eventually in principle all the material is converted to the crystalline diastereomer from the desired enantiomer. The desired enantiomer is then released from the diastereomer;

viii) kinetic resolutions - this technique refers to the achievement of partial or complete resolution of a racemate (or of a further resolution of a partially resolved compound) by virtue of unequal reaction rates of the enantiomers with a chiral, non-racemic reagent or catalyst under kinetic conditions;

ix) enantiospecific synthesis from non-racemic precursors - a synthetic technique whereby the desired enantiomer is obtained from non-chiral starting materials and where the stereochemical integrity is not or is only minimally compromised over the course of the synthesis;

x) chiral liquid chromatography - a technique whereby the enantiomers of a racemate are separated in a liquid mobile phase by virtue of their differing interactions with a stationary phase (including via chiral HPLC). The stationary phase can be made of chiral material or the mobile phase can contain an additional chiral material to provoke the differing interactions;

xi) chiral gas chromatography - a technique whereby the racemate is volatilized and enantiomers are separated by virtue of their differing interactions in the gaseous mobile phase with a column containing a fixed non-racemic chiral adsorbent phase;

xii) extraction with chiral solvents - a technique whereby the enantiomers are separated by virtue of preferential dissolution of one enantiomer into a particular chiral solvent;

xiii) transport across chiral membranes - a technique whereby a racemate is placed in contact with a thin membrane barrier. The barrier typically separates two miscible fluids, one containing the racemate, and a driving force such as concentration or pressure differential causes preferential transport across the membrane barrier. Separation occurs as a result of the non-racemic chiral nature of the membrane that allows only one enantiomer of the racemate to pass through.

Chiral chromatography, including simulated moving bed chromatography, is used in one embodiment. A wide variety of chiral stationary phases are commercially available.

Pharmaceutical Compositions

Pharmaceutical compositions based upon a compound of formula (I) or its pharmaceutically acceptable salt or prodrug can be prepared in a therapeutically effective amount for treating a *Flaviviridae* virus, optionally in combination with a pharmaceutically acceptable additive, carrier or excipient. The therapeutically effective amount may vary with the infection or condition to be treated, its severity, the treatment regimen to be employed, the pharmacokinetics of the agent used, as well as the patient treated.

In one aspect according to the present invention, the compound according to the present invention is formulated preferably in admixture with a pharmaceutically acceptable carrier. In general, it is preferable to administer the pharmaceutical composition in an intravenous form, but formulations may be prepared for administration via oral, parenteral, intramuscular, transdermal, buccal, subcutaneous, suppository or other route. Intravenous and intramuscular formulations are preferably administered in sterile saline. One of ordinary skill in the art may modify the formulation within the teachings of the specification to provide numerous formulations for a particular route. In particular, a modification of a desired compound to render it more soluble in water or other vehicle, for example, may be easily accomplished by routine modification (salt formulation, esterification, etc.).

In certain pharmaceutical dosage forms, for example an oral formuation, the prodrug form of the compound, especially including an acylated (acetylated or other) and ether derivative, phosphate ester or a salt forms of the present compound, is preferred. One of

ordinary skill in the art will recognize how to readily modify the present compound to a prodrug form to facilitate delivery of active compound to a targeted site within the host organism or patient. The artisan also will take advantage of favorable pharmacokinetic parameters of the prodrug form, where applicable, in delivering the desired compound to a targeted site within the host organism or patient to maximize the intended effect of the compound in the treatment of *Flaviviridae* (including HCV) infections.

The amount of compound included within therapeutically active formulations, according to the present invention, is an effective amount for treating a *Flaviviridae* (including HCV) infection.

Administration of the active compound may range from continuous (intravenous drip) to several oral administrations (for example, Q.I.D., B.I.D., etc.) and may include oral, topical, parenteral, intramuscular, intravenous, subcutaneous, transdermal (which may include a penetration enhancement agent), buccal and suppository administration, among other routes of administration. Enteric-coated oral tablets may also be used to enhance bioavailability and stability of the compounds from an oral route of administration. The most effective dosage form will depend upon the pharmacokinetics of the particular agent chosen, as well as the severity of disease in the patient.

In a first embodiment, the invention provides a method and composition for the treatment of a Flaviviridae infection, and in particular, a hepatitis C viral infection, that includes administering gemcitabine or its pharmaceutically acceptable salt or prodrug or derivative in a dosage range of approximately 50 mg/m^2 to about 1300 mg/ m^2 per day for one, two or three days, followed by cessation of therapy. In an alternative embodiment, for more severe Flaviviridae infections, gemcitabine or its pharmaceutically acceptable salt or prodrug or derivative is administered in a dosage range of approximately 50 mg/m^2 to about 1300 mg/ m^2 per day for between one and seven days (e.g., 1, 2, 3, 4, 5, 6, or 7 days), followed by cessation of therapy.

The daily dosage of gemcitabine or another active compound according to the invention can be selected to maximize the therapeutic effect. Examples of nonlimiting dosage ranges are between 100-1500 mg per day, alternatively between 200-1000 mg per day, and more particularly between 300-800 mg per day.

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compound as a pharmaceutically acceptable salt may be

appropriate. Pharmaceutically acceptable salts include those derived from pharmaceutically acceptable inorganic or organic bases and acids. Suitable salts include those derived from alkali metals such as potassium and sodium, alkaline earth metals such as calcium and magnesium, among numerous other acids well known in the pharmaceutical art. In particular, examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids, which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α-ketoglutarate, and α-glycerophosphate. Suitable inorganic salts may also be formed, including, sulfate, nitrate, bicarbonate, and carbonate salts. as well as hydrochloride and hydrobromide salts.

Any of the nucleosides described herein can be administered as a nucleotide prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the nucleoside. A number of nucleotide prodrug ligands are known. In general, alkylation, acylation or other lipophilic modification of the mono, di or triphosphate of the nucleoside will increase the stability of the nucleotide. Examples of substituent groups that can replace one or more hydrogens on the phosphate moiety are alkyl, aryl, steroids, carbohydrates, including sugars, 1,2-diacylglycerol and alcohols. Many are described in R. Jones and N. Bischofberger, *Antiviral Research*, 27 (1995) 1-17. Any of these can be used in combination with the disclosed nucleosides to achieve a desired effect.

The active nucleoside can also be provided as a 5'-phosphoether lipid or a 5' -ether lipid, as disclosed in the following references, which are incorporated by refer ence herein: Kucera, L.S., N. Iyer, E. Leake, A. Raben, Modest E.K., D.L.W., and C. Piantadosi. 1990. "Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation." *AIDS Res. Hum. Retro Viruses.* 6:491-501; Piantadosi, C., J. Marasco C.J., S.L. Morris-Natschke, K.L. Meyer, F. Gumus, J.R. Surles, K.S. Ishaq, L.S. Kucera, N. Iyer, C.A. Wallen, S. Piantadosi, and E.J. Modest. 1991. "Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV activity." *J. Med. Chem.* 34:1408.1414; Hosteller, K.Y., D.D. Richman, D.A. Carson, L.M. Stuhmiller, G.M. T. van Wijk, and H. van den Bosch. 1992. "Greatly enhanced inhibition of human immunodeficiency virus type 1 replication in CEM and HT4-6C cells by 3' -deoxythymidine diphosphate dimyristoylglycerol, a lipid prodrug of 3,-deoxythymidine." *Antimicrob. Agents Chemother.* 36:2025.2029; Hosetler, K.Y., L.M. Stuhmiller, H.B. Lenting, H. van den

Bosch, and D.D. Richman, 1990. "Synthesis and antiretroviral activity of phospholipid analogs of azidothymidine and other antiviral nucleosides." *J. Biol. Chem.* 265:61127.

Nonlimiting examples of U.S. patents that disclose suitable lipophilic substituents that can be covalently incorporated into the nucleoside, preferably at the 5'-OH position of the nucleoside or lipophilic preparations, include U.S. Patent Nos. 5,149,794 (Sep. 22, 1992, Yatvin *et al.*); 5,194,654 (Mar. 16, 1993, Hostetler *et al.*, 5,223,263 (June 29, 1993, Hostetler *et al.*); 5,256,641 (Oct. 26, 1993, Yatvin *et al.*); 5,411,947 (May 2, 1995, Hostetler *et al.*); 5,463,092 (Oct. 31, 1995, Hostetler *et al.*); 5,543,389 (Aug. 6, 1996, Yatvin *et al.*); 5,543,390 (Aug. 6, 1996, Yatvin *et al.*); 5,543,391 (Aug. 6, 1996, Yatvin *et al.*); and 5,554,728 (Sep. 10, 1996; Basava *et al.*), all of which are incorporated herein by reference. Foreign patent applications that disclose lipophilic substituents that can be attached to the nucleosides of the present invention, or lipophilic preparations, include WO 89/02733, W0 90/00555, W0 91/16920, W0 91/18914, W0 93/00910, W0 94/26273, W0 96/15132, EP 0 350 287, EP 93917054.4, and W0 91/19721.

To prepare the pharmaceutical compositions according to the present invention, a therapeutically effective amount of one or more of the compounds according to the present invention is preferably mixed with a pharmaceutically acceptable carrier according to conventional pharmaceutical compounding techniques to produce a dose. A carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravenous,or parenteral. In preparing pharmaceutical compositions in oral dosage form, any of the usual pharmaceutical media may be used. Thus, for liquid oral preparations such as suspensions, elixirs and solutions, suitable carriers and additives including water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like may be used. For solid oral preparations such as powders, tablets, capsules, and for solid preparations such as suppositories, suitable carriers and additives including starches, sugar carriers, such as dextrose, mannitol, lactose and related carriers, diluents, granulating agents, lubricants, binders, disintegrating agents and the like may be used. If desired, the tablets or capsules may be enteric-coated for sustained release by standard techniques. The use of these dosage forms may significantly impact the bioavailability of the compounds in the patient.

For parenteral formulations, the carrier will usually comprise sterile water or aqueous sodium chloride solution, though other ingredients, including those that aid dispersion, also may be included. Where sterile water is to be used and maintained as sterile, the compositions and carriers must also be sterilized. Injectable suspensions may also be

prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed.

Liposomal suspensions (including liposomes targeted to viral antigens) may also be prepared by conventional methods to produce pharmaceutically acceptable carriers. This may be appropriate for the delivery of free nucleosides, acyl nucleosides or phosphate ester prodrug forms of the nucleoside compounds according to the present invention.

In addition, the compounds according to the present invention can be administered in combination or alternation with one or more antiviral, anti-HIV, anti-HBV, anti-HCV or anti-herpetic agent or interferon, anti-cancer or antibacterial agents, including other compound. The preferred compounds include interferon alpha, ribavirin. Certain compounds according to the present invention may be effective for enhancing the biological activity of certain agents according to the present invention by reducing the metabolism, catabolism or inactivation of other compounds and as such, are co-administered for this intended effect.

In an additional embodiment, the method for the treatment or prophylaxis of a mammal having a virus-associated disorder which comprises administering to the mammal a pharmaceutically effective amount of gemcitabine, or its pharmaceutically acceptable salt or prodrug thereof, optionally in a combination or alternation with one or more other anti-virally effective agent(s), optionally in a pharmaceutically acceptable carrier or diluent, as disclosed herein, is provided. In a preferred embodiment, the mammal is a human.

In particular, the invention includes methods for treating or preventing and uses for the treatment or prophylaxis of a *Flaviviridae* infection, including all members of the Hepacivirus genus (HCV), Pestivirus genus (BVDV, CSFV, BDV), or Flavivirus genus (Dengue virus, Japanese encephalitis virus group (including West Nile Virus), and Yellow Fever virus).

This invention is further illustrated in the following sections. The Examples contained therein are set forth to aid in an understanding of the invention. This section is not intended to, and should not be interpreted to, limit in any way the invention set forth in the claims that follow thereafter.

Therapies for the Treatment of *Flaviviridae* Infection

It has been recognized that drug-resistant variants of viruses can emerge after prolonged treatment with an antiviral agent. Drug resistance most typically occurs by

mutation of a gene that encodes for an enzyme used in the viral replication cycle, and most typically in the case of HCV, the RNA-dependent-RNA polymerase. It has been demonstrated that the efficacy of a drug against viral infection can be prolonged, augmented, or restored by administering the compound in combination or alternation with a second, and perhaps third, antiviral compound that induces a different mutation from that caused by the principle drug. Alternatively, the pharmacokinetics, biodistribution or other parameter of the drug can be altered by such combination or alternation therapy. In general, combination therapy is typically preferred over alternation therapy because it induces multiple simultaneous stresses on the virus.

Examples of agents that have been identified as active against Flaviviridae, and in particular the hepatitis C virus, and thus can be used in combination or alternation with gemcitabine, its salt, prodrug or derivative are described in the following numbered paragraphs.

(1) interferon and/or ribavirin.

(2) Substrate-based NS3 protease inhibitors (Attwood *et al.*, *Antiviral peptide derivatives*, PCT WO 98/22496, **1998**; Attwood *et al.*, *Antiviral Chemistry and Chemotherapy* **1999**, *10*, 259-273; Attwood *et al.*, *Preparation and use of amino acid derivatives as anti-viral agents,* German Patent Pub. DE 19914474; Tung *et al. Inhibitors of serine proteases, particularly hepatitis C virus NS3 protease*, PCT WO 98/17679), including alphaketoamides and hydrazinoureas, and inhibitors that terminate in an electrophile such as a boronic acid or phosphonate (Llinas-Brunet et al, *Hepatitis C inhibitor peptide analogues,* PCT WO 99/07734).

(3) Non-substrate-based inhibitors such as 2,4,6-trihydroxy-3-nitro-benzamide derivatives (Sudo K. *et al.*, *Biochemical and Biophysical Research Communications*, **1997**, *238*, 643-647; Sudo K. *et al. Antiviral Chemistry and Chemotherapy*, **1998**, *9*, 186), including RD3-4082 and RD3-4078, the former substituted on the amide with a 14 carbon chain and the latter processing a *para*-phenoxyphenyl group.

(4) Thiazolidine derivatives which show relevant inhibition in a reverse-phase HPLC assay with an NS3/4A fusion protein and NS5A/5B substrate (Sudo K. *et al.*, *Antiviral Research*, **1996**, *32*, 9-18), especially compound RD-1-6250, possessing a fused cinnamoyl moiety substituted with a long alkyl chain, RD4 6205 and RD4 6193.

(5) Thiazolidines and benzanilides identified in Kakiuchi N. *et al. J. EBS Letters 421*, 217-220; Takeshita N. *et al. Analytical Biochemistry*, **1997**, *247*, 242-246.

(6) A phenan-threnequinone possessing activity against protease in a SDS-PAGE and autoradiography assay isolated from the fermentation culture broth of *Streptomyces* sp., Sch 68631 (Chu M. *et al., Tetrahedron Letters*, **1996**, *37*, 7229-7232), and Sch 351633, isolated from the fungus *Penicillium griscofuluum,* which demonstrates activity in a scintillation proximity assay (Chu M. *et al., Bioorganic and Medicinal Chemistry Letters 9*, 1949-1952).

(7) Selective NS3 inhibitors based on the macromolecule elgin c, isolated from leech (Qasim M.A. *et al., Biochemistry*, **1997**, *36*, 1598-1607).

(8) Helicase inhibitors (Diana G.D. *et al., Compounds, compositions and methods for treatment of hepatitis C*, U.S. Pat. No. 5,633,358; Diana G.D. *et al., Piperidine derivatives, pharmaceutical compositions thereof and their use in the treatment of hepatitis C*, PCT WO 97/36554).

(9) Polymerase inhibitors such as nucleotide analogues, gliotoxin (Ferrari R. *et al. Journal of Virology*, **1999**, *73*, 1649-1654), and the natural product cerulenin (Lohmann V. *et al., Virology*, **1998**, *249*, 108-118).

(10) Antisense phosphorothioate oligodeoxynucleotides (S-ODN) complementary to sequence stretches in the 5' non-coding region (NCR) of the virus (Alt M. *et al., Hepatology*, **1995**, *22*, 707-717), or nucleotides 326-348 comprising the 3' end of the NCR and nucleotides 371-388 located in the core coding region of the HCV RNA (Alt M. *et al., Archives of Virology*, **1997**, *142*, 589-599; Galderisi U. *et al., Journal of Cellular Physiology*, **1999**, *181*, 251-257).

(11) Inhibitors of IRES-dependent translation (Ikeda N *et al., Agent for the prevention and treatment of hepatitis C,* Japanese Patent Pub. JP-08268890; Kai Y. *et al. Prevention and treatment of viral diseases,* Japanese Patent Pub. JP-10101591).

(12) Nuclease-resistant ribozymes (Maccjak, D. J. *et al., Hepatology* **1999**, *30*, abstract 995).

(13) Nucleoside analogs have also been developed for the treatment of Flaviviridae infections.

(14) Idenix Pharmaceuticals, Ltd. discloses branched nucleosides, and their use in the treatment of HCV and flaviviruses and pestiviruses in International Publication Nos. WO

01/90121 (filed May 23, 2001) and WO 01/92282 (filed May 26, 2001). A method for the treatment of hepatitis C infection (and flaviviruses and pestiviruses) in humans and other host animals is disclosed in the Idenix publications that includes administering an effective amount of a biologically active 1', 2', 3' or 4'-branched β-D or β-L nucleosides or a pharmaceutically acceptable salt or prodrug thereof, administered either alone or in combination, optionally in a pharmaceutically acceptable carrier.

(15) WO 01/96353 (filed June 15, 2001) to Indenix Pharmaceuticals, Ltd. discloses 3'-prodrugs of 2'-deoxy-β-L-nucleosides for the treatment of HBV. U.S. Patent No. 4,957,924 to Beauchamp discloses various therapeutic esters of acyclovir.

(16) Other patent applications disclosing the use of certain nucleoside analogs to treat hepatitis C virus include: PCT/CA00/01316 (WO 01/32153; filed November 3, 2000) and PCT/CA01/00197 (WO 01/60315; filed February 19, 2001) filed by BioChem Pharma, Inc. (now Shire Biochem, Inc.); PCT/US02/01531 (WO 02/057425; filed January 18, 2002) and PCT/US02/03086 (WO 02/057287; filed January 18, 2002) filed by Merck & Co., Inc., PCT/EP01/09633 (WO 02/18404; published August 21, 2001) filed by Roche, and PCT Publication No. WO 01/79246 (filed April 13, 2001) and WO 02/32920 (filed October 18, 2001) by Pharmasset.

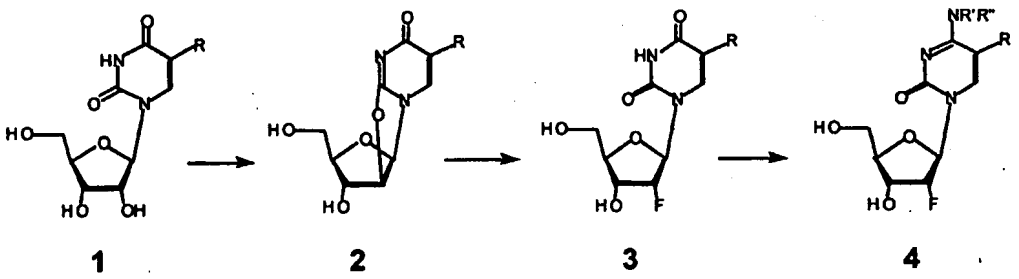
(17) Other miscellaneous compounds including 1-amino-alkylcyclohexanes (U.S. Patent No. 6,034,134 to Gold *et al.*), alkyl lipids (U.S. Pat. No. 5,922,757 to Chojkier *et al.*), vitamin E and other antioxidants (U.S. Pat. No. 5,922,757 to Chojkier *et al.*), squalene, amantadine, bile acids (U.S. Pat. No. 5,846,964 to Ozeki *et al.*), N-(phosphonoacetyl)-L-aspartic acid, (U.S. Pat. No. 5,830,905 to Diana *et al.*), benzenedicarboxamides (U.S. Pat. No. 5,633,388 to Diana *et al.*), polyadenylic acid derivatives (U.S. Pat. No. 5,496,546 to Wang *et al.*), 2',3'-dideoxyinosine (U.S. Pat. No. 5,026,687 to Yarchoan *et al.*), and benzimidazoles (U.S. Pat. No. 5,891,874 to Colacino *et al.*).

(18) Other compounds currently in clinical development for treatment of hepatitis c virus include: Interleukin-10 by Schering-Plough, IP-501 by Interneuron, Merimebodib VX-497 by Vertex, AMANTADINE (Symmetrel) by Endo Labs Solvay, HEPTAZYME by RPI, IDN-6556 by Idun Pharma., XTL-002 by XTL., HCV/MF59 by Chiron, CIVACIR by NABI, LEVOVIRIN by ICN, VIRAMIDINE by ICN, ZADAXIN (thymosin alfa-1) by Sci Clone, CEPLENE (histamine dihydrochloride) by Maxim, VX 950 / LY 570310 by Vertex/Eli Lilly, ISIS 14803 by Isis Pharmaceutical/Elan, IDN-6556 by Idun Pharmaceuticals, Inc. and JTK 003 by AKROS Pharma.

(19) U.S. Patent No. 6,348,587 to Emory University and the University of Georgia Research Foundation discloses the use of 2'-fluoronucleosides for the treatment of HIV, hepatitis B, hepatitis C and abnormal cellular proliferation.

Synthetic Protocol

For pyrimidine nucleosides, uridine derivative (**1**, Scheme 1) is the starting material, which is converted into 2,2'-anhydro derivative (**2**) which is treated with HF in anhydrous dioxane (Codington *et al., J Org. Chem.*, **1964**, *29*, 558). The corresponding 2'-fluoro-2'-deoxyuridine derivative (**3**) is obtained in 40-50% yield. Modification at the 4 position in **3** can be achieved by various methods. 2'-Fluoro-2'-deoxycytidine derivatives (**4**, R = R' = R" = H) can be readily prepared from **3** by the well-known procedures via thiation or chlorination.

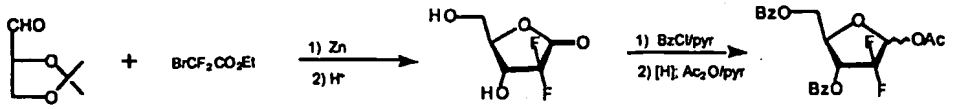


1 **2** **3** **4**

Scheme 1. Synthesis of 2'-fluoro-2'-deoxy-uridine and cytidine derivatives.

Starting from L-uridine, all the L-nucleoside counterparts synthesized in the D-series can be prepared.

gem-Difluoronucleosides can be obtained by condensation of 2,2-difluoro-1-O-acetyl-3,5-di-O-benzoyl-2-deoxo-D-ribofuranos-2-ulose (**8**, Scheme 2) with various silyated pyrimidine bases or with purines by the sodium salt method. The sugar can be readily prepared from 2,3-O-isopropylidene-D-glyceral (**5**) and ethyl bromodifluoroacetate (**6**) by Reformatzky reaction, followed by acidic removal of protecting groups to give lactone **7**. Benzoylation of 7, and subsequent conversion of the lactone to lactol by DIBAL reduction and acetylation affords **8**.



5 6 7 8

Scheme 2. Preparation of 2,2-difluoro-sugar synthesis for nucleoside synthesis.

Examples

The following working examples provide a further understanding of the method of the present invention. These examples are of illustrative purposes, and are not meant to limit the scope of the invention. Equivalent, similar or suitable solvents, reagents or reaction conditions may be substituted for those particular solvents, reagents or reaction conditions described without departing from the general scope of the method

10

Example 1

Antiviral testing of candidate compounds for Flaviviridae: The HCV replicon system in Huh7 cells. Huh7 cells harboring the HCV replicon can be cultivated in DMEM media (high glucose, no pyruvate) containing 10% fetal bovine serum, 1X non-essential Amino Acids, Pen-Strep-Glu (100 units/liter, 100 microgram/liter, and 2.92 mg/liter, respectively) and 500 to 1000 microgram/milliliter G418. Antiviral screening assays can be done in the same media without G418 as follows: in order to keep cells in logarithmic growth phase, seed cells in a 96-well plate at low density, for example 1000 cells per well. Add the test compound immediate after seeding the cells and incubate for a period of 3 to 7 days at 37°C
20 in an incubator. Media is then removed, and the cells are prepared for total nucleic acid extraction (including replicon RNA and host RNA). Replicon RNA can then be amplified in a Q-RT-PCR protocol, and quantified accordingly. The observed differences in quantification of replicon RNA is one way to express the antiviral potency of the test compound. A typical experiment demonstrates that in the negative control and in the non-active compounds-settings a comparable amount of replicon is produced. This can be concluded because the measured threshold-cycle for HCV RT-PCR in both setting is close to each other. In such experiments, one way to express the antiviral effectiveness of a compound is to subtract the threshold RT-PCR cycle of the test compound with the average threshold RT-PCR cycle of the negative control. This value is called DeltaCt (ΔCt or DCt).

A ΔCt of 3.3 equals a 1-log reduction (equals EC_{90}) in replicon production. Compounds that result in a reduction of HCV replicon RNA levels of greater than 2 □Ct values (75% reduction of replicon RNA) are candidate compounds for antiviral therapy. Such candidate compounds are belonging to structures with general formula (I). As a positive control, recombinant interferon alfa-2a (Roferon-A, Hoffmann-Roche, New Jersey, USA) is taken alongside as positive control.

However, this HCV ΔCt value does not include any specificity parameter for the replicon encoded viral RNA-dependent RNA polymerase. In a typical setting, a compound might reduce both the host RNA polymerase activity and the replicon-encoded polymerase activity. Therefore, quantification of rRNA (or any other host RNA polymerase I product) or beta-actin mRNA (or any other host RNA polymerase II) and comparison with RNA levels of the no-drug control is a relative measurement of the effect of the test compound on host RNA polymerases.

With the availability of both the HCV ΔCt data and the rRNA ΔCt, a specificity parameter can be introduced. This parameter is obtained by subtracting both ΔCt values from each other. This results in Delta-DeltaCT values (ΔΔCt or DDCt); a value above 0 means that there is more inhibitory effect on the replicon encoded polymerase, a ΔCt value below 0 means that the host rRNA levels are more affected than the replicon levels. As a general rule, ΔCt values above 2 are considered as significantly different from the no-drug treatment control, and hence, exhibits appreciable antiviral activity. However, compounds with a ΔCt value of less than 2, but showing limited molecular cytotoxicty data (rRNA ΔCT between 0 and 2) are also possible active compounds.

In another typical setting, a compound might reduce the host RNA polymerase activity, but not the host DNA polymerase activity. Therefore, quantification of rDNA or beta-actin DNA (or any other host DNA fragment) and comparison with DNA levels of the no-drug control is a relative measurement of the inhibitory effect of the test compound on cellular DNA polymerases

With the availability of both the HCV □Ct data and the rDNA □Ct, a specificity parameter can be introduced. This parameter is obtained by subtracting both □Ct values from each other. This results in ΔΔCt values; a value above 0 means that there is more inhibitory effect on the replicon encoded polymerase, a ΔΔCt value below 0 means that the host rDNA levels are more affected than the replicon levels. As a general rule, ΔΔCt values

above 2 are considered as significantly different from the no-drug treatment control, and hence, is an interested compound for further evaluation. However, compounds with a ΔΔCt value of less than 2, but with limited molecular cytotoxicty (rDNA ΔCT between 0 and 2) may be desired.

Compounds that result in the specific reduction of HCV replicon RNA levels, but with limited reductions in cellular RNA and/or DNA levels are candidate compounds for antiviral therapy. Candidate compounds belonging to general formula group (I) were evaluated for their specific capacity of reducing Flaviviridae RNA (including HCV), and potent compounds were detected.

Most studies indicate that HCV genotypes 1a and 1b are more resistant to treatment with any interferon alpha-based therapy than non-type 1 genotypes. For this reason, some doctors may prescribe longer durations of treatment for patients infected with viral genotypes 1a or 1b. Therefore, in one embodiment, gemcitabine is administered to a patient infected with HCV1a or 1b in doses effective in reducing viral load. Therefore, in one embodiment of the invention, gemcitadine is administered to a host carrying HCV genotype 1a or 1b independently of interferon alpha. In a further embodiment, gemcitabine is administered to a host carrying HCV genotype 1a or 1b in combination with interferon alpha.

EXAMPLE 2: Antiviral Activity of Gemcitabine (dFdC)

Gemcitabine was dissolved in DMSO and added to the culture media of a cellular model system of Huh7 cells harboring self-replicating HCV RNA, at final concentrations ranging from 0.1 to 50 dM. In such experiments, one way to express the antiviral effectiveness of a compound is to subtract the threshold reverse-transcriptase polymerase chain reactions (RT-PCR) cycle of the test compound with the average threshold RT-PCR cycle of the negative control. This value is called DeltaCt (ΔCt or dCt). With the availability of both the HCV ΔCt data and the rRNA ΔCt, a specificity parameter can be introduced. This parameter is obtained by subtracting both ΔCt values from each other. This results in Delta-DeltaCT values (ΔΔCt or ddCt). A 4-days incubation resulted in dose-dependant reduction of the replicon HCV RNA (**Figure 2**). Since 3.3 Ct values equals 1-log reduction of replicon RNA, an EC_{90} value was reached at approximately 70 nM. Further analysis of the reduction of cellular DNA levels (ribosomal DNA) or cellular RNA levels (ribosomal RNA) resulted in

a dCt that expressed the inhibitory capacity of this compound on host DNA and RNA polymerases. Based on these calculations, In a cellular model system of Huh7 cells harboring self-replicating HCV RNA, gemcitabine significantly reduced HCV RNA levels (EC_{50} = 0.040 μM) at a concentration below the IC_{50} (0.240 μM). Interestingly, the inactive metabolite dFdU (7.0 μM) demonstrated similar activity to dFdC in the HCV replicon system [dCT HCV = 6.39, dCt rRNA = 1.96, and ddCt: 4.42; (EC_{50} and IC_{50} data not available)].

EXAMPLE 3: Antiviral activity of gemcitabine after single treatment in human

A male patient exhibiting multifocal HCC, cirrhosis, and ischaemic hepatitis infected with HCV was administered 1200 mg gemcitabine HCl in 1000 minutes associated with oxaliplatine. The tolerance was acceptable, and thus the next day the patient was given a second dosage of approximately 700 mg of gemcitabine. Before the second dosage the baseline viral load was 6.49 log copies/mL. The second perfusion of gemcitabine was stopped after approximately 700 mg because of heart problems. The HCV RNA measurement eight hours after the second dosage was 4.04 log copies/mL, indicating an approximate 2.5 log drop in eight hours.

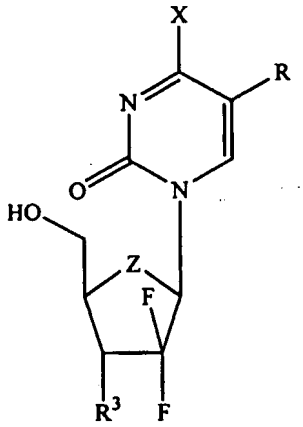
I claim:

1. A method for the treatment of a patient infected with a hepatitis C virus, comprising administering gemcitabine or its pharmaceutically acceptable salt or prodrug
   (i) in an amount between 50-1300 mg/m^2 of host surface area
   (ii) in a dosage regimen of daily for one, two, three., four, five, six or seven consecutive days followed by cessation of therapy.
2. The method of claim 1 wherein gemcitabine or its salt or prodrug is administered in an amount between 200 – 1000 mg/m^2 per day.
3. The method of claim 1 wherein gemcitabine is administered in an amount between 300 – 800 mg/m^2 per day.
4. The method of claim 1 wherein the dosage regimen is once a day for one day.
5. The method of claim 1 wherein the dosage regimen is once a day for two days.
6. The method of claim 1 wherein the dosage regimen is once a day for three days.
7. The method of claim 1 wherein the dosage regimen is once a day for four days.
8. The method of claim 1 wherein the dosage regimen is once a day for five days.
9. The method of claim 1 wherein the dosage regimen is once a day for six days.
10. The method of claim 1 wherein the dosage regimen is once a day for seven days.
11. The method of claim 1 wherein the dosage is administered intravenously.
12. The method of claim 1, wherein the therapy is ceased for at least two days.
13. The method of claim 1, wherein the therapy is ceased for at least three days.
14. The method of claim 1, wherein the therapy is ceased for at least one week.
15. The method of claim 1, wherein the therapy is ceased for at least two weeks.
16. The method of claim 1, wherein the therapy is ceased for at least three weeks.
17. The method of claim 1, wherein the therapy is ceased for at least one month.

18. A method for the treatment of a patient infected with a Flaviviridae infection , comprising administering gemcitabine or its pharmaceutically acceptable salt or prodrug
   (iii) in an amount between 50-1300 mg/m^2 of host surface area
   (iv) in a dosage regimen of daily for one, two, three., four, five, six or seven consecutive days followed by cessation of therapy.
19. The method of claim 18 wherein gemcitabine or its salt or prodrug is administered in an amount between 200 – 1000 mg/m^2 per day.

20. The method of claim 18 wherein gemcitabine is administered in an amount between 300 – 800 mg/m^2 per day.
21. The method of claim 18 wherein the dosage regimen is once a day for one day.
22. The method of claim 18 wherein the dosage regimen is once a day for two days.
23. The method of claim 18 wherein the dosage regimen is once a day for three days.
24. The method of claim 18 wherein the dosage regimen is once a day for four days.
25. The method of claim 18 wherein the dosage regimen is once a day for five days.
26. The method of claim 18 wherein the dosage regimen is once a day for six days.
27. The method of claim 18 wherein the dosage regimen is once a day for seven days.
28. The method of claim 18 wherein the dosage is administered intravenously.
29. The method of claim 18 wherein the therapy is ceased for at least two days.
30. The method of claim 18, wherein the therapy is ceased for at least three days.
31. The method of claim 18, wherein the therapy is ceased for at least one week.
32. The method of claim 18, wherein the therapy is ceased for at least two weeks.
33. The method of claim 18, wherein the therapy is ceased for at least three weeks.
34. The method of claim 18, wherein the therapy is ceased for at least one month.

35. A method for the treatment of *Flaviviridae* virus, comprising administering an antivirally effective amount of a β-D or β-L nucleoside of the structure:



or a pharmaceutically acceptable salt or prodrug, in combination with one or more other antivirally effective agents

(i) in an amount between 50-1300 mg/m^2 of host surface area

(ii) in a dosage regimen of daily for one, two, three., four, five, six or seven consecutive days followed by cessation of therapy. in an amount between 50-1300 mg/m^2

wherein:

R is H, halogen (F, Cl, Br, I), OH, OR', SH, SR', NH_2, NHR', NR'$_2$, lower alkyl of C_1-C_6, halogenated (F, Cl, Br, I) lower alkyl of C_1-C_6 such as CF_3 and CH_2CH_2F, lower alkenyl of C_2-C_6 such as $CH=CH_2$, halogenated (F, Cl, Br, I) lower alkenyl of C_2-C_6 such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C_2-C_6 such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C_2-C_6, lower alkoxy of C_1-C_6 such as CH_2OH and CH_2CH_2OH, CO_2H, CO_2R', $CONH_2$, CONHR', CONR'$_2$, $CH=CHCO_2H$, $CH=CHCO_2R'$;

X and is independently H, halogen, OH, OR', OCH_3, SH, SR', SCH_3, NH_2, NHR', NR'$_2$, CH_3;

each R' is independently a hydrogen, lower alkyl of C_1-C_6 or lower cycloalkyl of C_1-C_6;

Z is O, S or CH_2; and

R^3 is F or OH.

36. The method of claim 35 wherein X is NH_2, Z is O, R^3 is OH, and R is H.
37. The method of claim 35 wherein gemcitabine or its salt or prodrug is administered in an amount between 200 – 1000 mg/m^2 per day.
38. The method of claim 35 wherein gemcitabine is administered in an amount between 300 – 800 mg/m^2 per day.
39. The method of claim 35 wherein the dosage regimen is once a day for one day.
40. The method of claim 35 wherein the dosage regimen is once a day for two days.
41. The method of claim 35 wherein the dosage regimen is once a day for three days.
42. The method of claim 35 wherein the dosage regimen is once a day for four days.
43. The method of claim 35 wherein the dosage regimen is once a day for five days.
44. The method of claim 35 wherein the dosage regimen is once a day for six days.
45. The method of claim 35 wherein the dosage regimen is once a day for seven days.
46. The method of claim 35 wherein the dosage is administered intravenously.
47. The method of claim 35, wherein the therapy is ceased for at least two days.
48. The method of claim 35, wherein the therapy is ceased for at least three days.

49. The method of claim 35, wherein the therapy is ceased for at least one week.
50. The method of claim 35, wherein the therapy is ceased for at least two weeks.
51. The method of claim 35, wherein the therapy is ceased for at least three weeks.
52. The method of claim 35, wherein the therapy is ceased for at least one month.
53. The method of claim 35, wherein the Flaviviridae is hepatitis C virus.
54. The method of claim 18 or 35, wherein the Flaviviridae is West Nile Virus.
55. The method of claim 18 or 35, wherein the Flaviviridae is Dengue virus.
56. The method of claim 18 or 35, wherein the Flaviviridae is Bovine Viral Diarrhea Virus.
57. The method of claim 18 or 35, wherein the Flaviviridae is Border Disease Virus.
58. The method of claim 18 or 35, wherein the Flaviviridae is Yellow Fever virus.
59. Use of gemcitabine or its pharmaceutically acceptable salt or prodrug
    (v) in an amount between 50-1300 mg/m^2 of host surface area
    (vi) in a dosage regimen of daily for one, two, three., four, five, six or seven consecutive days followed by cessation of therapy.

    in the treatment of a patient infected with a hepatitis C virus.
60. The use of claim 59 wherein gemcitabine or its salt or prodrug is administered in an amount between 200 – 1000 mg/m^2 per day.
61. The use of claim 59 wherein gemcitabine is administered in an amount between 300 – 800 mg/m^2 per day.
62. The use of claim 59 wherein the dosage regimen is once a day for one day.
63. The use of claim 59 wherein the dosage regimen is once a day for two days.
64. The use of claim 59 wherein the dosage regimen is once a day for three days.
65. The use of claim 59 wherein the dosage regimen is once a day for four days.
66. The use of claim 59 wherein the dosage regimen is once a day for five days.
67. The use of claim 59 wherein the dosage regimen is once a day for six days.
68. The use of claim 59 wherein the dosage regimen is once a day for seven days.
69. The use of claim 59 wherein the dosage is administered intravenously.
70. The use of claim 59, wherein the therapy is ceased for at least two days.
71. The use of claim 59, wherein the therapy is ceased for at least three days.
72. The use of claim 59, wherein the therapy is ceased for at least one week.
73. The use of claim 59, wherein the therapy is ceased for at least two weeks.
74. The use of claim 59, wherein the therapy is ceased for at least three weeks.
75. The use of claim 59, wherein the therapy is ceased for at least one month.

76. Use of gemcitabine or its pharmaceutically acceptable salt or prodrug

(vii) in an amount between 50-1300 mg/m^2 of host surface area

(viii) in a dosage regimen of daily for one, two, three., four, five, six or seven consecutive days followed by cessation of therapy

for the treatment of a patient infected with a Flaviviridae infection.

77. The use of claim 76 wherein gemcitabine or its salt or prodrug is administered in an amount between 200 – 1000 mg/m^2 per day.

78. The use of claim 76 wherein gemcitabine is administered in an amount between 300 – 800 mg/m^2 per day.

79. The use of claim 76 wherein the dosage regimen is once a day for one day.

80. The use of claim 76 wherein the dosage regimen is once a day for two days.

81. The use of claim 76 wherein the dosage regimen is once a day for three days.

82. The use of claim 76 wherein the dosage regimen is once a day for four days.

83. The use of claim 76 wherein the dosage regimen is once a day for five days.

84. The use of claim 76 wherein the dosage regimen is once a day for six days.

85. The use of claim 76 wherein the dosage regimen is once a day for seven days.

86. The use of claim 76 wherein the dosage is administered intravenously.

87. The use of claim 76 wherein the therapy is ceased for at least two days.

88. The use of claim 76, wherein the therapy is ceased for at least three days.

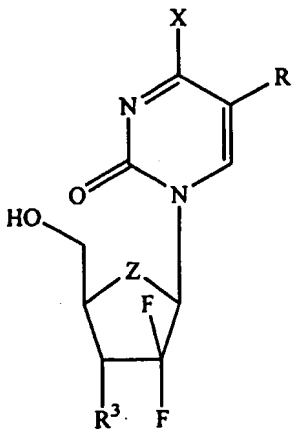
89. The use of claim 76, wherein the therapy is ceased for at least one week.

90. The use of claim 76, wherein the therapy is ceased for at least two weeks.

91. The use of claim 76, wherein the therapy is ceased for at least three weeks.

92. The use of claim 76, wherein the therapy is ceased for at least one month.

93. Use of an antivirally effective amount of a β-D or β-L nucleoside of the structure:



or a pharmaceutically acceptable salt or prodrug, in combination with one or more other antivirally effective agents

(i) in an amount between 50-1300 mg/m^2 of host surface area

(ii) in a dosage regimen of daily for one, two, three., four, five, six or seven consecutive days followed by cessation of therapy. in an amount between 50-1300 mg/m^2

for the treatment of *Flaviviridae* virus,

wherein:

R is H, halogen (F, Cl, Br, I), OH, OR', SH, SR', NH_2, NHR', NR'$_2$, lower alkyl of C_1-C_6, halogenated (F, Cl, Br, I) lower alkyl of C_1-C_6 such as CF_3 and CH_2CH_2F, lower alkenyl of C_2-C_6 such as $CH=CH_2$, halogenated (F, Cl, Br, I) lower alkenyl of C_2-C_6 such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C_2-C_6 such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C_2-C_6, lower alkoxy of C_1-C_6 such as CH_2OH and CH_2CH_2OH, CO_2H, CO_2R', $CONH_2$, CONHR', CONR'$_2$, $CH=CHCO_2H$, $CH=CHCO_2R'$;

X and is independently H, halogen, OH, OR', OCH_3, SH, SR', SCH_3, NH_2, NHR', NR'$_2$, CH_3;

each R' is independently a hydrogen, lower alkyl of C_1-C_6 or lower cycloalkyl of C_1-C_6;

Z is O, S or CH_2; and

R^3 is F or OH.

94. The use of claim 93 wherein X is NH_2, Z is O, R^3 is OH, and R is H.

95. The use of claim 93 wherein gemcitabine or its salt or prodrug is administered in an amount between 200 – 1000 mg/m^2 per day.

96. The use of claim 93 wherein gemcitabine is administered in an amount between 300 – 800 mg/m^2 per day.

97. The use of claim 93 wherein the dosage regimen is once a day for one day.

98. The method of claim 93 wherein the dosage regimen is once a day for two days.

99. The method of claim 93 wherein the dosage regimen is once a day for three days.

100. The use of claim 93 wherein the dosage regimen is once a day for four days.

101. The use of claim 93 wherein the dosage regimen is once a day for five days.

102. The use of claim 93 wherein the dosage regimen is once a day for six days.

103. The use of claim 93 wherein the dosage regimen is once a day for seven days.

104. The use of claim 93 wherein the dosage is administered intravenously.

105. The use of claim 93, wherein the therapy is ceased for at least two days.

106. The use of claim 93, wherein the therapy is ceased for at least three days.

107. The use of claim 93, wherein the therapy is ceased for at least one week.

108. The use of claim 93, wherein the therapy is ceased for at least two weeks.

109. The use of claim 93, wherein the therapy is ceased for at least three weeks.

110. The use of claim 93, wherein the therapy is ceased for at least one month.

111. The use of claim 93, wherein the Flaviviridae is hepatitis C virus.

112. The use of claim 76 or 93, wherein the Flaviviridae is West Nile Virus.

113. The use of claim 76 or 93, wherein the Flaviviridae is Dengue virus.

114. The use of claim 76 or 93, wherein the Flaviviridae is Bovine Viral Diarrhea Virus.

115. The use of claim 76 or 93, wherein the Flaviviridae is Border Disease Virus.

116. The use of claim 76 or 93, wherein the Flaviviridae is Yellow Fever virus.

117. Use of gemcitabine or its pharmaceutically acceptable salt or prodrug

(ix) in an amount between 50-1300 mg/m^2 of host surface area

(x) in a dosage regimen of daily for one, two, three., four, five, six or seven consecutive days followed by cessation of therapy

in the manufacture of a medicament for the treatment of a patient infected with a Flaviviridae infection.

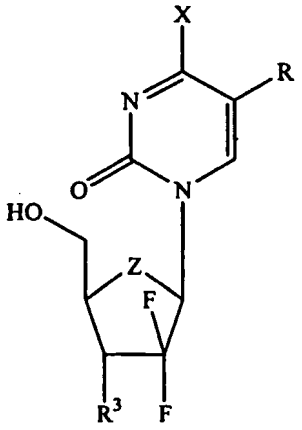
118. Use of gemcitabine or its pharmaceutically acceptable salt or prodrug

(xi) in an amount between 50-1300 mg/m^2 of host surface area

(xii) in a dosage regimen of daily for one, two, three., four, five, six or seven consecutive days followed by cessation of therapy

in the manufacture of a medicament for the treatment of a patient infected with a Flaviviridae infection.

119. Use of an antivirally effective amount of a β-D or β-L nucleoside of the structure:



or a pharmaceutically acceptable salt or prodrug, in combination with one or more other antivirally effective agents

(i) in an amount between 50-1300 mg/m^2 of host surface area

(ii) in a dosage regimen of daily for one, two, three., four, five, six or seven consecutive days followed by cessation of therapy. in an amount between 50-1300 mg/m^2

in the manufacture of a medicament for the treatment of *Flaviviridae* virus, wherein:

R is H, halogen (F, Cl, Br, I), OH, OR', SH, SR', NH_2, NHR', NR'$_2$, lower alkyl of C_1-C_6, halogenated (F, Cl, Br, I) lower alkyl of C_1-C_6 such as CF_3 and CH_2CH_2F, lower alkenyl of C_2-C_6 such as $CH=CH_2$, halogenated (F, Cl, Br, I) lower alkenyl of C_2-C_6 such as CH=CHCl, CH=CHBr and CH=CHI, lower alkynyl of C_2-C_6 such as C≡CH, halogenated (F, Cl, Br, I) lower alkynyl of C_2-C_6, lower alkoxy of C_1-C_6 such as CH_2OH and CH_2CH_2OH, CO_2H, CO_2R', $CONH_2$, CONHR', CONR'$_2$, $CH=CHCO_2H$, $CH=CHCO_2R'$;

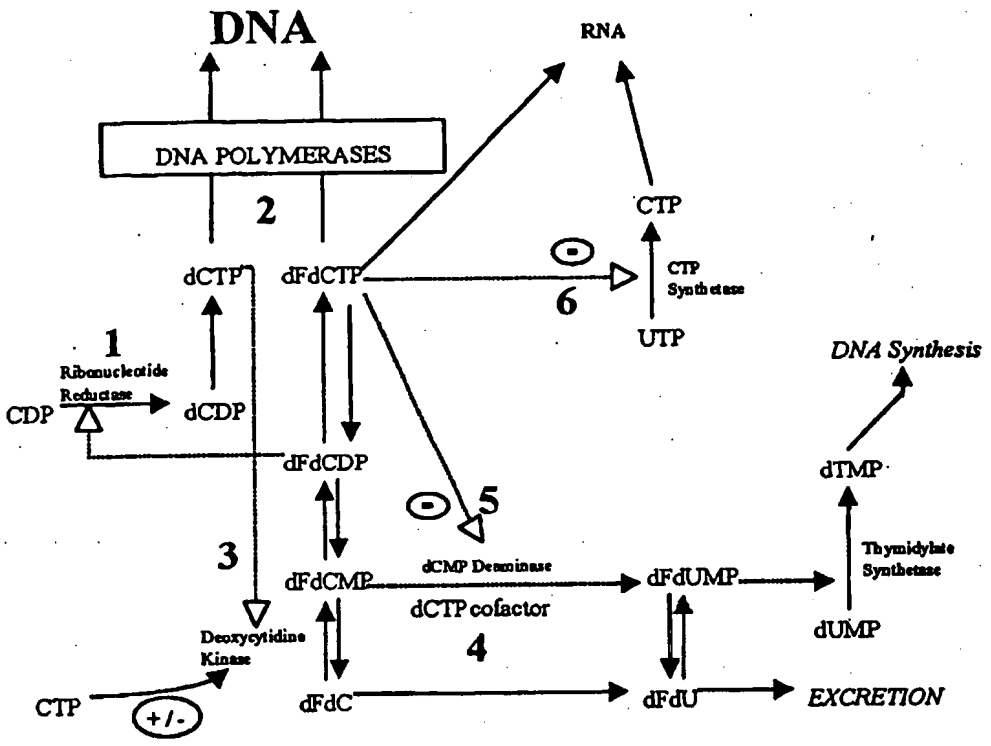
X and is independently H, halogen, OH, OR', OCH_3, SH, SR', SCH_3, NH_2, NHR', NR'$_2$, CH_3;

each R' is independently a hydrogen, lower alkyl of C_1-C_6 or lower cycloalkyl of C_1-C_6;

Z is O, S or CH_2; and

R^3 is F or OH.

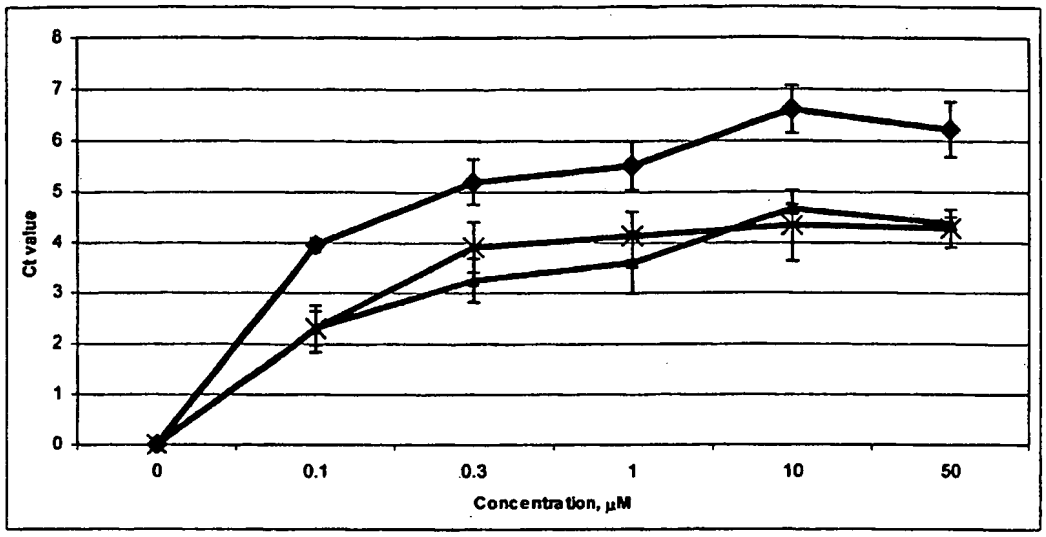
Figure 1. Self-Potentiating Actions of Gemcitabine



1) dFdCDP inhibits ribonucleotide reductase 2) reductions in dCTP favor dFdCTP incorporation into DNA 3) reductions in dCTP increase dCK activity and increased FdCMP formation 4) reductions in dCTP, a positive cofactor for dCMP deaminase activity, reduces dFdCMP deamination 5) dFdCTP inhibits dCMP deaminase 6) dFdCTP inhibits CTP synthetase

Figure 2: Anti HCV activity of Gemcitabine (dFdC)

♦: ΔCt for HCV RNA, ▲: HCV-rDNA ΔΔCt; X: HCV-rRNA ΔΔCt



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(54) Title: METHODS OF INHIBITING ORTHOPOXVIRUS REPLICATION WITH NUCLEOSIDE COMPOUNDS

(57) Abstract: The present invention provides methods of inhibiting orthopoxvirus replication and/or treating orthopoxvirus infection with certain nucleoside compounds and derivatives thereof. These compounds are particularly useful as inhibitors of vaccinia virus and variola virus replication and/or for the treatment of vaccinia virus and variola virus infection. The nucleoside compounds may be administered alone or in combination with other agents active against orthopoxvirus infection, in particular against vaccinia virus or variola virus infection. Another aspect of the present invention provides for the use of such nucleoside compounds in the manufacture of a medicament for the inhibition of orthopoxvirus replication and/or for the treatment of orthopoxvirus infection. Yet a further aspect of the present invention provides such nucleoside compounds for use as a medicament for the inhibition of orthopoxvirus replication and/or for the treatment of orthopoxvirus infection.

TITLE OF THE INVENTION

METHODS OF INHIBITING ORTHOPOXVIRUS REPLICATION WITH NUCLEOSIDE COMPOUNDS

FIELD OF THE INVENTION

The present invention is concerned with methods of inhibiting orthopoxvirus replication and methods for treating orthopoxvirus infections with certain nucleoside compounds and derivatives thereof. The compounds are particularly useful for inhibiting the replication of vaccinia, variola, cowpox, and monkeypox virus and for the treatment of vaccinia, variola, cowpox, and monkeypox virus infections. Another aspect of the present invention provides for the use of the nucleoside compounds and derivatives thereof and their pharmaceutical compositions for the manufacture of a medicament for the inhibition of orthopoxvirus replication and/or for the treatment of orthopoxvirus infection. Yet a further aspect of the present invention provides for the nucleoside compounds and derivatives thereof and their pharmaceutical compositions for use as a medicament for the inhibition of orthopoxvirus replication and/or for the treatment of orthopoxvirus infection.

BACKGROUND OF THE INVENTION

Orthopoxvirus is a genus of the *Poxviridae* family of complex DNA viruses that replicate in the cytoplasm of vertebrate and invertebrate cells. The Poxviridae family is characterized by having a large complex virion containing enzymes that synthesize mRNA, a genome composed of a single linear double-stranded DNA molecule of 130-300 kilobase pairs with a hairpin loop at each end, and a cytoplasmic site of replication. Members of the orthopoxvirus genus include cowpox, monkeypox, vaccinia, and variola virus [for a description of the *Poxviridae* family, reference is made to B. Moss, "Poxviridae: The Viruses and Their Replication," in Fields Virology, B.N. Fields, et al., Eds., 3rd ed., Ch. 83, pages 2637-2671 (1996)]. Variola virus is the agent responsible for smallpox infections. Smallpox infections were effectively eradicated subsequent to the introduction of prophylactic vaccinations with cowpox and vaccinia virus. However, most of the human populations worldwide are no longer immune to smallpox as a result of the discontinuation of routine vacination in the early 1980's.

There are very few compounds available as therapeutics against orthopoxvirus infections. Two drugs under investigation are cidofovir and ribavirin.

Cidofovir is the generic name for (S)-1-[3-hydroxy-2-(phosphonylmethoxy)-propyl]cytosine [(S)-HPMPC] which is currently the leading agent for the treatment of orthopoxvirus infections in humans. It is potent against vaccinia and cowpox virus infection in mice when administered subcutaneously or intraperitoneally. However, cidofovir's therapeutic utility is limited by safety concerns as well as lack of oral bioavailablity [see D.F. Smee et al., "Effects of cidofovir on the pathogenesis of a lethal vaccinia virus respiratory infection in mice," Antiviral Res., 52: 55-62 (2001) and references cited therein]. Inhibition of vaccinia virus is considered in the art to be predictive of inhibitory activity against other orthopoxviruses, including variola; see E. De Clercq, "Vaccinia Virus Inhibitors as a Paradigm for the Chemotherapy of Poxvirus Infections," Clin. Microbiol. Rev., 14: 382-397 (2001).

Ribavirin has also been found to inhibit vaccinia virus and other orthopoxvirus replication in cell culture (see J.H. Huffman et al., "In vitro effect of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide on DNA and RNA viruses," Antimicrobial Agents and Chemotherapy, 3: 235-241 (1973) and D.F. Smee et al., "Potential of the IMP dehydrogenase inhibitors for antiviral therapies of poxvirus infections," Antiviral Res., 37: A89 (1998)]. Ribavirin was also reported to suppress vaccinia virus-induced lesions in a mouse model and to effectively treat vaccinia keratitis in rabbits. However, ribavirin causes anemia during prolonged administration and at high doses has certain immunosuppressive properties limiting its clinical usefulness against orthopoxvirus.

Consequently, there exists a need for more effective anti-orthopoxvirus agents particularly as a result of the threat of either variola (smallpox) or monkeypox viruses in biowarfare or bioterrorism. Preferably such agents should be effective when administered orally and be safe and well-tolerated by the host.

It has now been found that nucleoside compounds of the present invention and certain derivatives thereof are potent inhibitors of orthopoxvirus replication and in particular of vaccinia, variola, cowpox, and monkeypox virus replication. The instant nucleoside compounds and derivatives thereof are useful to treat orthopoxvirus infection and in particular vaccinia, variola, cowpox, and monkeypox virus infection.

It is therefore an object of the present invention to provide nucleoside compounds and certain derivatives thereof which are useful as inhibitors of the replication of orthopoxvirus and in particular as inhibitors of the replication of vaccinia, variola, cowpox, and monkeypox virus.

It is another object of the present invention to provide nucleoside compounds and certain derivatives thereof which are useful in the treatment of orthopoxvirus infection and in particular in the treatment of vaccinia, variola, cowpox, and monkeypox virus infection.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds of the present invention in association with a pharmaceutically acceptable carrier.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds and derivatives of the present invention for use as inhibitors of orthopoxvirus replication and in particular as inhibitors of vaccinia, variola, cowpox, and monkeypox virus replication.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds and derivatives of the present invention for use in the treatment of orthopoxvirus infection and in particular in the treatment of vaccinia, variola, cowpox, and monkeypox virus infection.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds and derivatives of the present invention in combination with other agents active against orthopoxvirus and in particular against vaccinia, variola, cowpox, and monkeypox virus.

It is another object of the present invention to provide methods for the inhibition of orthopoxvirus replication and in particular for the inhibition of vaccinia, variola, cowpox, and monkeypox virus replication.

It is another object of the present invention to provide methods for the treatment of orthopoxvirus infection and in particular for the treatment of vaccinia, variola, cowpox, and monkeypox virus infection.

It is another object of the present invention to provide methods for the treatment of orthopoxvirus infection in combination with other agents active against orthopoxvirus and in particular for the treatment of vaccinia, variola, cowpox, and monkeypox virus infection in combination with other agents active against vaccinia, variola, cowpox, and monkeypox virus infection.

It is another object of the present invention to provide nucleoside compounds and certain derivatives thereof and their pharmaceutical compositions for use as a medicament for the inhibition of orthopoxvirus replication and/or the treatment of orthopoxvirus infection and in particular for the inhibition of vaccinia,

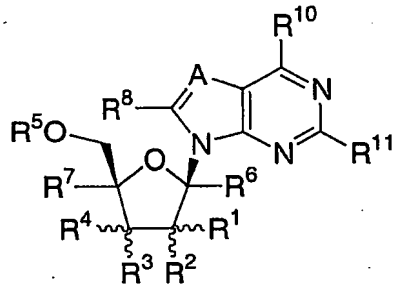
variola, cowpox, and monkeypox virus replication and/or the treatment of vaccinia, variola, cowpox, and monkeypox virus infection.

It is another object of the present invention to provide for the use of the nucleoside compounds and certain derivatives thereof of the present invention and their pharmaceutical compositions for the manufacture of a medicament for the inhibition of orthopoxvirus replication and/or the treatment of orthopoxvirus infection and in particular for the inhibition of vaccinia, variola, cowpox, and monkeypox virus replication and/or the treatment of vaccinia, variola, cowpox, and monkeypox virus infection.

These and other objects will become readily apparent from the detailed description which follows.

SUMMARY OF THE INVENTION

The present invention provides a method for inhibiting orthopoxvirus replication and/or a method for treating orthopoxvirus infection in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of a compound of structural formula I:



(I)

or a pharmaceutically acceptable salt thereof; wherein

A is N or C-R^9;

R^1 is C_{2-4} alkenyl, C_{2-4} alkynyl, or C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^2 is amino, fluorine, hydroxy, C_{1-10} alkylcarbonyloxy, mercapto, or C_{1-4} alkoxy;

R^3 and R^4 are each independently selected from the group consisting of hydrogen, cyano, azido, halogen, hydroxy, C_{1-16} alkylcarbonyloxy, C_{2-18} alkenylcarbonyloxy, C_{1-10} alkyloxycarbonyloxy, C_{3-6} cycloalkylcarbonyloxy, C_{3-6} cycloalkyloxycarbonyloxy, mercapto, amino, C_{1-4} alkoxy, C_{2-4} alkenyl, C_{2-4} alkynyl, and C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^5 is hydrogen, C_{1-16} alkylcarbonyl, C_{2-18} alkenylcarbonyl, C_{1-10} alkyloxycarbonyl, C_{3-6} cycloalkylcarbonyl, C_{3-6} cycloalkyloxycarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or $P(O)R^{13}R^{14}$;

R^6 and R^7 are each independently hydrogen, methyl, hydroxymethyl, or fluoromethyl;

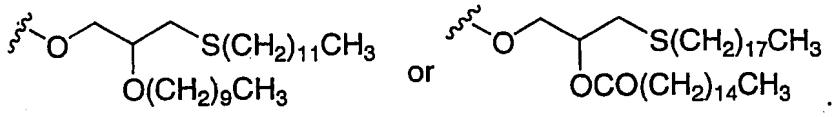
R^8 is hydrogen, C_{1-4} alkyl, C_{2-4} alkynyl, halogen, cyano, carboxy, C_{1-4} alkyloxycarbonyl, azido, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, hydroxy, C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfonyl, or (C_{1-4} alkyl)$_{0-2}$ aminomethyl;

R^9 is hydrogen, cyano, nitro, $NHCONH_2$, $CONR^{12}R^{12}$, $CSNR^{12}R^{12}$, $COOR^{12}$, $C(=NH)NH_2$, hydroxy, C_{1-3} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, halogen, or C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with one to three groups independently selected from halogen, amino, hydroxy, carboxy, and C_{1-3} alkoxy;

R^{10} and R^{11} are each independently hydrogen, hydroxy, mercapto, halogen, C_{1-4} alkoxy, C_{1-4} alkylthio, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{3-6} cycloalkylamino, di(C_{3-6} cycloalkyl)amino, phenyl-C_{1-2} alkylamino, C_{1-4} acylamino, C_{1-8} alkylcarbonyloxy, or $OCH(C_{1-4}$ alkyl$)O(C=O)C_{1-4}$ alkyl;

each R^{12} is independently hydrogen or C_{1-6} alkyl; and

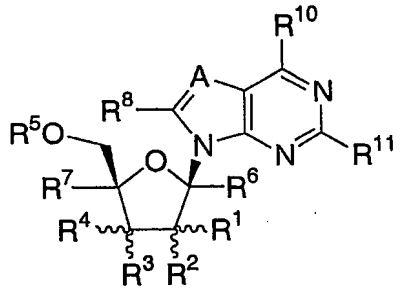
R^{13} and R^{14} are each independently hydroxy, $OCH_2CH_2SC(=O)C_{1-4}$ alkyl, $OCH_2O(C=O)OC_{1-4}$ alkyl, $NHCHMeCO_2Me$, $OCH(C_{1-4}$ alkyl$)O(C=O)C_{1-4}$ alkyl,



Also encompassed within the present invention are pharmaceutical compositions containing the compounds alone or in combination with other agents active against orthopoxvirus and in particular against vaccinia, variola, cowpox, and monkeypox virus as well as methods for the inhibition of orthopoxvirus replication and for the treatment of orthopoxvirus infection with such compositions.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for inhibiting orthopoxvirus replication and/or a method for treating orthopoxvirus infection in a mammal in need thereof comprising administering to the mammal a therapeutically effective amount of a compound of structural formula I:



(I)

or a pharmaceutically acceptable salt thereof; wherein

A is N or C-R^9;

R^1 is C_{2-4} alkenyl, C_{2-4} alkynyl, or C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^2 is amino, fluorine, hydroxy, C_{1-10} alkylcarbonyloxy, mercapto, or C_{1-4} alkoxy;

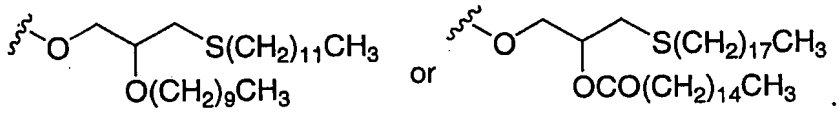
R^3 and R^4 are each independently selected from the group consisting of hydrogen, cyano, azido, halogen, hydroxy, C_{1-16} alkylcarbonyloxy, C_{2-18} alkenylcarbonyloxy, C_{1-10} alkyloxycarbonyloxy, C_{3-6} cycloalkylcarbonyloxy, C_{3-6} cycloalkyloxycarbonyloxy, mercapto, amino, C_{1-4} alkoxy, C_{2-4} alkenyl, C_{2-4} alkynyl, and C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^5 is hydrogen, C_{1-16} alkylcarbonyl, C_{2-18} alkenylcarbonyl, C_{1-10} alkyloxycarbonyl, C_{3-6} cycloalkylcarbonyl, C_{3-6} cycloalkyloxycarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or $P(O)R^{13}R^{14}$;

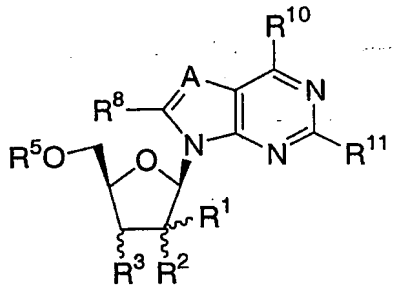
R^6 and R^7 are each independently hydrogen, methyl, hydroxymethyl, or fluoromethyl;

R^8 is hydrogen, C_{1-4} alkyl, C_{2-4} alkynyl, halogen, cyano, carboxy, C_{1-4} alkyloxycarbonyl, azido, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, hydroxy,

C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfonyl, or (C_{1-4} alkyl)$_{0-2}$ aminomethyl;
R^9 is hydrogen, cyano, nitro, $NHCONH_2$, $CONR^{12}R^{12}$, $CSNR^{12}R^{12}$, $COOR^{12}$, $C(=NH)NH_2$, hydroxy, C_{1-3} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, halogen, or C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with one to three groups independently selected from halogen, amino, hydroxy, carboxy, and C_{1-3} alkoxy;
R^{10} and R^{11} are each independently hydrogen, hydroxy, mercapto, halogen, C_{1-4} alkoxy, C_{1-4} alkylthio, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{3-6} cycloalkylamino, di(C_{3-6} cycloalkyl)amino, phenyl-C_{1-2} alkylamino, C_{1-4} acylamino, C_{1-8} alkylcarbonyloxy, or $OCH(C_{1-4}$ alkyl$)O(C=O)C_{1-4}$ alkyl;
each R^{12} is independently hydrogen or C_{1-6} alkyl; and
R^{13} and R^{14} are each independently hydroxy, $OCH_2CH_2SC(=O)C_{1-4}$ alkyl, $OCH_2O(C=O)OC_{1-4}$ alkyl, $NHCHMeCO_2Me$, $OCH(C_{1-4}$ alkyl$)O(C=O)C_{1-4}$ alkyl,



In one embodiment of the present invention is the method of inhibiting orthopoxvirus replication and/or treating orthopoxvirus infection with a compound of structural formula II which is of the stereochemical configuration:



(II)

or a pharmaceutically acceptable salt thereof; wherein
A is N or C-R^9;
R^1 is C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-3} alkoxy, C_{1-3} alkylthio, or one to three fluorine atoms;

R^2 is hydroxy, C_{1-16} alkylcarbonyloxy, fluoro, or C_{1-3} alkoxy;
R^3 is hydrogen, halogen, hydroxy, C_{1-16} alkylcarbonyloxy, amino, or C_{1-3} alkoxy;
R^5 is hydrogen, C_{1-16} alkylcarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or PO_3H_2;
R^8 is hydrogen, amino, or C_{1-4} alkylamino;
R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and
R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino.

In a second embodiment of the present invention is the method of inhibiting orthopoxvirus replication and/or treating orthopoxvirus infection with a compound of structural formula II wherein
R^1 is methyl, fluoromethyl, hydroxymethyl, difluoromethyl, trifluoromethyl, or aminomethyl;
R^2 is hydroxy, C_{1-16} alkylcarbonyloxy, fluoro, or methoxy;
R^3 is hydrogen, fluoro, hydroxy, C_{1-16} alkylcarbonyloxy, amino, or methoxy;
R^5 is hydrogen, C_{1-16} alkylcarbonyl, or $P_3O_9H_4$;
R^8 is hydrogen or amino;
R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and
R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino.

Illustrative of the invention is a method for inhibiting orthopoxvirus replication and/or treating orthopox infection wherein the compound is selected from:
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-methylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-dimethylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile,
4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2,4-diamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-hydroxypurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-cyclopropylaminopurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2,6-diaminopurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-methylaminopurine,
6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine,
2'-*C*-methyl-adenosine,
4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine, and
4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

Further illustrative of the invention is a method for inhibiting orthopoxvirus replication and/or treating orthopox infection wherein the compound is selected from:
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine,
2'-*C*-methyl-adenosine,
4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine, and

4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

In a third embodiment of the methods of the present invention, the orthopoxvirus replication is selected from the group consisting of vaccinia virus replication, variola virus replication, cowpox virus replication, and monkeypox virus replication. In a class of this embodiment, the orthopoxvirus replication is vaccinia virus replication or variola virus replication.

In a fourth embodiment of the methods of the present invention, the orthopoxvirus infection is selected from the group consisting of vaccinia virus infection, variola virus infection, cowpox virus infection, and monkeypox virus infection. In a class of this embodiment, the orthopoxvirus infection is vaccinia virus infection or variola virus infection.

Another aspect of the present invention provides the novel nucleoside derivative, 6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine or a pharmaceutically acceptable salt thereof.

Throughout the instant application, the following terms have the indicated meanings:

The alkyl groups specified above are intended to include those alkyl groups of the designated length in either a straight or branched configuration. Exemplary of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tertiary butyl, pentyl, isopentyl, hexyl, isohexyl, and the like.

The term "alkenyl" shall mean straight or branched chain alkenes of two to six total carbon atoms, or any number within this range (e.g., ethenyl, propenyl, butenyl, pentenyl, etc.).

The term "alkynyl" shall mean straight or branched chain alkynes of two to six total carbon atoms, or any number within this range (e.g., ethynyl, propynyl, butynyl, pentynyl, etc.).

The term "cycloalkyl" shall mean cyclic rings of alkanes of three to eight total carbon atoms, or any number within this range (i.e., cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, or cyclooctyl).

The term "alkoxy" refers to straight or branched chain alkoxides of the number of carbon atoms specified (e.g., C_{1-4} alkoxy), or any number within this range [i.e., methoxy (MeO-), ethoxy, isopropoxy, etc.].

The term "alkylthio" refers to straight or branched chain alkylsulfides of the number of carbon atoms specified (e.g., C_{1-4} alkylthio), or any number within this range [i.e., methylthio (MeS-), ethylthio, isopropylthio, etc.].

The term "alkylamino" refers to straight or branched alkylamines of the number of carbon atoms specified (e.g., C_{1-4} alkylamino), or any number within this range [i.e., methylamino, ethylamino, isopropylamino, t-butylamino, etc.].

The term "alkylsulfonyl" refers to straight or branched chain alkylsulfones of the number of carbon atoms specified (e.g., C_{1-6} alkylsulfonyl), or any number within this range [i.e., methylsulfonyl ($MeSO_2$-), ethylsulfonyl, isopropylsulfonyl, etc.].

The term "alkyloxycarbonyl" refers to straight or branched chain esters of a carboxylic acid derivative of the present invention of the number of carbon atoms specified (e.g., C_{1-4} alkyloxycarbonyl), or any number within this range [i.e., methyloxycarbonyl (MeOCO-), ethyloxycarbonyl, or butyloxycarbonyl].

The term "alkyloxycarbonyloxy" refers to straight or branched chain alkyl carbonates of the present invention of the number of carbon atoms specified (e.g., C_{1-10} alkyloxycarbonyloxy), or any number within this range [i.e., methyloxycarbonyloxy (MeOCOO-), ethyloxycarbonyloxy, or butyloxycarbonyloxy].

The term "alkylcarbonyloxy" refers to straight or branched chain alkanoic acid derivatives of alcohols of the present invention of the number of carbon atoms specified (e.g., C_{1-16} alkylcarbonyloxy), or any number within this range [i.e., methylcarbonyloxy (MeCOO-), ethylcarbonyloxy, or n-octylcarbonyloxy].

The term "cycloalkylcarbonyloxy" refers to cyclic alkanoic acid derivatives of alcohols of the present invention of the number of carbon atoms specified (e.g., C_{3-6} cycloalkylcarbonyloxy), or any number within this range [i.e., cyclopropylcarbonyloxy, cyclopentylcarbonyloxy, or cyclohexylcarbonyloxy].

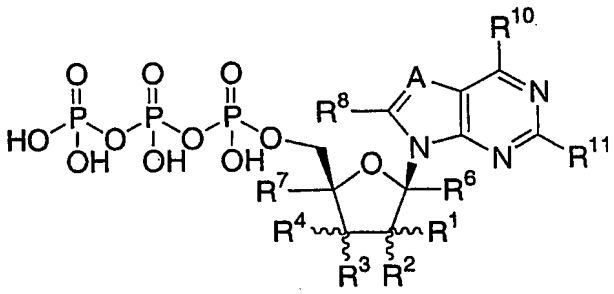
The term "alkenylcarbonyloxy" refers to a straight or branched chain alkenoic acid derivatives of alcohols of the present invention having two to eighteen total carbon atoms and containing one to three double bonds in the alkene chain.

The term "aryl" includes both phenyl, naphthyl, and pyridyl. The aryl group is optionally substituted with one to three groups independently selected from C_{1-4} alkyl, halogen, cyano, nitro, trifluoromethyl, C_{1-4} alkoxy, and C_{1-4} alkylthio.

The term "halogen" is intended to include the halogen atoms fluorine, chlorine, bromine and iodine.

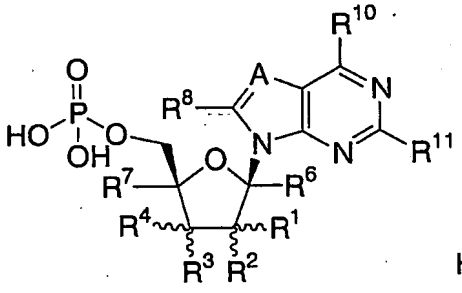
The term "substituted" shall be deemed to include multiple degrees of substitution by a named substituent. Where multiple substituent moieties are disclosed or claimed, the substituted compound can be independently substituted by one or more of the disclosed or claimed substituent moieties, singly or plurally.

The term "5'-triphosphate" refers to a triphosphoric acid ester derivative of the 5'-hydroxyl group of a nucleoside compound of the present invention having the following general structural formula III:

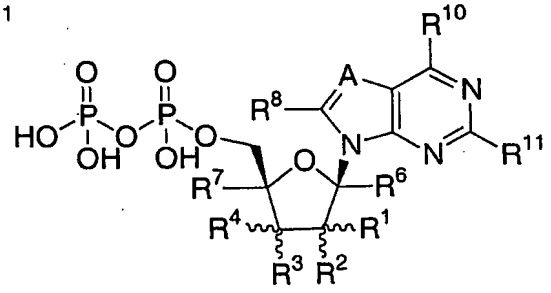


(III)

wherein R^1-R^{11} are as defined above. The compounds of the present invention are also intended to include pharmaceutically acceptable salts of the triphosphate ester as well as pharmaceutically acceptable salts of 5'-monophosphate and 5'-diphosphate ester derivatives of the structural formulae IV and V, respectively,

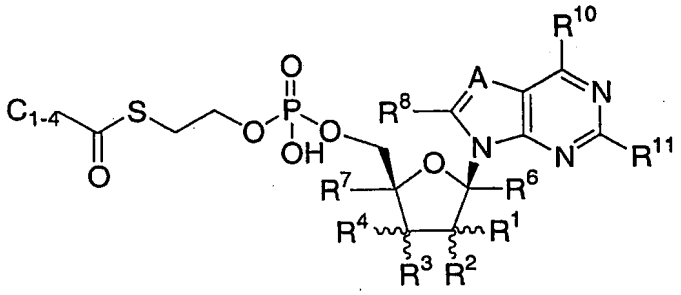


(IV)

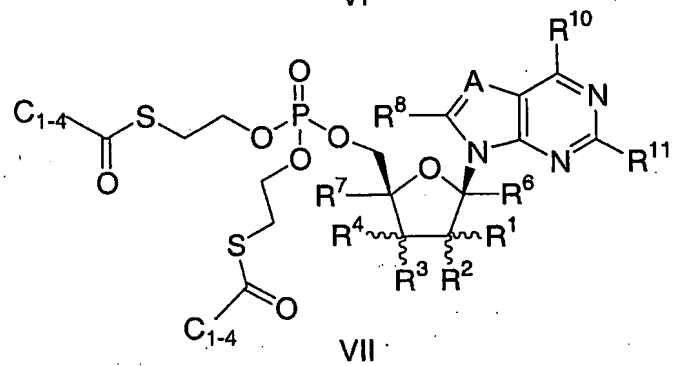


(V)

The term "5'-(S-acyl-2-thioethyl)phosphate" or "SATE" refers to a mono- or di-ester derivative of a 5'-monophosphate nucleoside derivative of the present invention of structural formulae VI and VII, respectively, as well as pharmaceutically acceptable salts of the mono-ester,



VI



VII

The term "composition", as in "pharmaceutical composition," is intended to encompass a product comprising the active ingredient(s) and the inert ingredient(s) that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound of the present invention and a pharmaceutically acceptable carrier.

The terms "administration of" and "administering a" compound should be understood to mean providing a compound of the invention or a prodrug of a compound of the invention to the individual in need.

Another aspect of the present invention is concerned with a method of treating orthopoxvirus infections with a compound of the present invention in combination with one or more agents useful for treating orthopoxvirus infections. Such agents active against orthopoxviruses include, but are not limited to, cidofovir, ribavirin, levovirin, and viramidine. Levovirin is the L-enantiomer of ribavirin which has shown immunomodulatory activity similar to ribavirin. Viramidine represents an analog of ribavirin disclosed in WO 01/60379 (assigned to ICN Pharmaceuticals). In accordance with this method of the present invention, the individual components of the combination can be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. The instant invention is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment, and the term "administering" is to be interpreted accordingly. It will be understood that the scope of combinations of the compounds of this invention with other agents useful for treating orthopoxvirus infection includes in principle any combination with any pharmaceutical composition for treating orthopoxvirus infection. When a compound of the present invention or a pharmaceutically acceptable salt thereof is used in combination with a second therapeutic agent active against orthopoxvirus, the dose of each compound may be either the same as or different from the dose when the compound is used alone.

Ribavirin, levovirin, and viramidine may exert their anti-orthopoxvirus effects by modulating intracellular pools of guanine nucleotides via inhibition of the intracellular enzyme inosine monophosphate dehydrogenase (IMPDH). IMPDH is the rate-limiting enzyme on the biosynthetic route in *de novo* guanine nucleotide biosynthesis. Ribavirin is readily phosphorylated intracellularly and the monophosphate derivative is an inhibitor of IMPDH. Thus, inhibition of IMPDH represents another useful target for the discovery of inhibitors of orthopoxvirus replication. Therefore, the compounds of the present invention may also be administered in combination with an inhibitor of IMPDH, such as VX-497, which is disclosed in WO 97/41211 and WO 01/00622 (assigned to Vertex); another IMPDH inhibitor, such as that disclosed in WO 00/25780 (assigned to Bristol-Myers Squibb); or mycophenolate mofetil [see A.C. Allison and E.M. Eugui, Agents Action, 44 (Suppl.): 165 (1993)].

By "pharmaceutically acceptable" is meant that the carrier, diluent, or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Also included within the present invention are pharmaceutical compositions comprising the nucleoside compounds and derivatives thereof of the present invention in association with a pharmaceutically acceptable carrier. Another example of the invention is a pharmaceutical composition made by combining any of the compounds described above and a pharmaceutically acceptable carrier. Another illustration of the invention is a process for making a pharmaceutical composition comprising combining any of the compounds described above and a pharmaceutically acceptable carrier.

Also included within the present invention are pharmaceutical compositions useful for inhibiting orthopoxvirus replication comprising an effective amount of a compound of the present invention and a pharmaceutically acceptable carrier. Pharmaceutical compositions useful for treating orthopoxvirus infection are also encompassed by the present invention. Additionally, the present invention is directed to a pharmaceutical composition comprising a therapeutically effective amount of a compound of the present invention in combination with a therapeutically effective amount of another agent active against orthopoxvirus. Agents active against orthopoxvirus include, but are not limited to, cidofovir, ribavirin, levovirin, and viramidine.

Another aspect of the present invention provides for the use of the nucleoside compounds and derivatives thereof and their pharmaceutical compositions for the manufacture of a medicament for the inhibition of orthopoxvirus replication, in particular vaccinia virus, variola virus replication, cowpox virus replication, and monkeypox virus replication and/or the treatment of orthopoxvirus infection, in particular vaccinia virus, variola virus infection, cowpox virus infection, and monkeypox virus infection. Yet a further aspect of the present invention provides for the nucleoside compounds and derivatives thereof and their pharmaceutical compositions for use as a medicament for the inhibition of orthopoxvirus replication, in particular vaccinia, variola, cowpox, and monkeypox virus replication, and/or for the treatment of orthopoxvirus infection, in particular vaccinia, variola, cowpox, and monkeypox virus infection.

The pharmaceutical compositions of the present invention comprise a compound of structural formula I as an active ingredient or a pharmaceutically

acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients.

The compositions include compositions suitable for oral, rectal, topical, parenteral (including subcutaneous, intramuscular, and intravenous), ocular (ophthalmic), pulmonary (nasal or buccal inhalation), or nasal administration, although the most suitable route in any given case will depend on the nature and severity of the conditions being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the art of pharmacy.

In practical use, the compounds of structural formula I can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, hard and soft capsules and tablets, with the solid oral preparations being preferred over the liquid preparations.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques. Such compositions and preparations should contain at least 0.1 percent of active compound. The percentage of active compound in these compositions may, of course, be varied and may conveniently be between about 2 percent to about 60 percent of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that an effective dosage will be obtained. The active compounds can also be administered intranasally as, for example, liquid drops or spray.

The tablets, pills, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid; a

lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin. When a dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as a fatty oil.

Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain, in addition to the active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

Compounds of structural formula I may also be administered parenterally. Solutions or suspensions of these active compounds can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g. glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

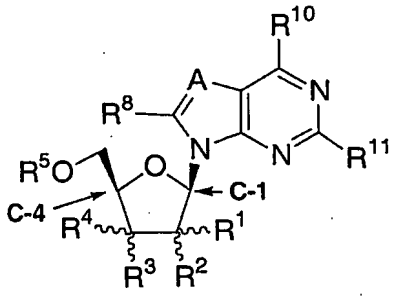
Any suitable route of administration may be employed for providing a mammal, especially a human with an effective dosage of a compound of the present invention. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, and the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, and the like. Preferably compounds of structural formula I are administered orally.

For oral administration to humans, the dosage range is 0.01 to 1000 mg/kg body weight in divided doses. In one embodiment the dosage range is 0.1 to 100 mg/kg body weight in divided doses. In another embodiment the dosage range is 0.5 to 20 mg/kg body weight in divided doses. For oral administration, the compositions are preferably provided in the form of tablets or capsules containing 1.0 to 1000 milligrams of the active ingredient, particularly, 1, 5, 10, 15, 20, 25, 50, 75,

100, 150, 200, 250, 300, 400, 500, 600, 750, 800, 900, and 1000 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated.

The effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration, the condition being treated and the severity of the condition being treated. Such dosage may be ascertained readily by a person skilled in the art. This dosage regimen may be adjusted to provide the optimal therapeutic response.

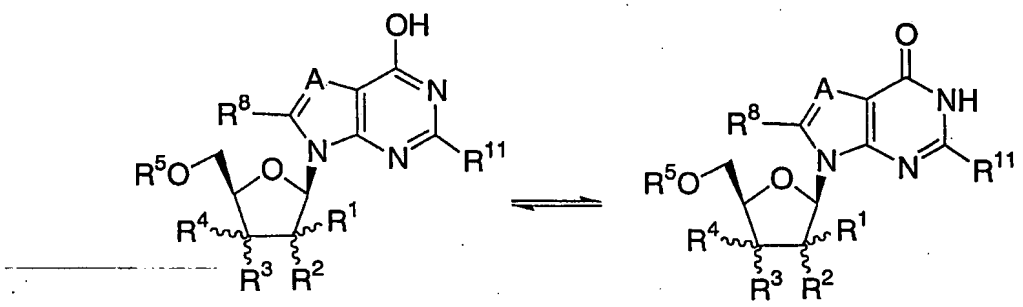
The compounds of the present invention contain one or more asymmetric centers and can thus occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. The present invention is meant to comprehend nucleoside compounds having the β-D stereochemical configuration for the five-membered furanose ring as depicted in the structural formula below, that is, nucleoside compounds in which the substituents at C-1 and C-4 of the five-membered furanose ring have the β-stereochemical configuration ("up" orientation as denoted by a bold line).



β-D-

Some of the compounds described herein contain olefinic double bonds, and unless specified otherwise, are meant to include both E and Z geometric isomers.

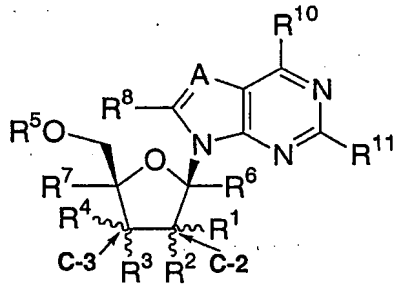
Some of the compounds described herein may exist as tautomers such as keto-enol tautomers. The individual tautomers as well as mixtures thereof are encompassed with compounds of structural formula I. An example of keto-enol tautomers which are intended to be encompassed within the compounds of the present invention is illustrated below:



Compounds of structural formula I may be separated into their individual diastereoisomers by, for example, fractional crystallization from a suitable solvent, for example methanol or ethyl acetate or a mixture thereof, or via chiral chromatography using an optically active stationary phase.

Alternatively, any stereoisomer of a compound of the structural formula I may be obtained by stereospecific synthesis using optically pure starting materials or reagents of known configuration.

The stereochemistry of the substituents at the C-2 and C-3 positions of the furanose ring of the compounds of the present invention of structural formula I is denoted by squiggly lines which signifies that substituents R^1, R^2, R^3 and R^4 can have either the α (substituent "down") or β (substituent "up") configuration independently of one another. Notation of stereochemistry by a bold line as at C-1 and C-4 of the furanose ring signifies that the substituent has the β-configuration (substituent "up").



(I)

The compounds of the present invention may be administered in the form of a pharmaceutically acceptable salt. The term "pharmaceutically acceptable salt" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids. Salts of

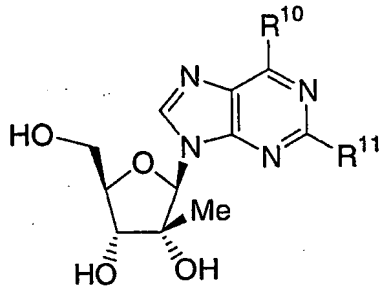
basic compounds encompassed within the term "pharmaceutically acceptable salt" refer to non-toxic salts of the compounds of this invention which are generally prepared by reacting the free base with a suitable organic or inorganic acid. Representative salts of basic compounds of the present invention include, but are not limited to, the following: acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, sulfate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide and valerate. Furthermore, where the compounds of the invention carry an acidic moiety, suitable pharmaceutically acceptable salts thereof include, but are not limited to, salts derived from inorganic bases including aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic, mangamous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, cyclic amines, and basic ion-exchange resins, such as arginine, betaine, caffeine, choline, N,N-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

Also, in the case of a carboxylic acid (-COOH) or alcohol group being present in the compounds of the present invention, pharmaceutically acceptable esters of carboxylic acid derivatives, such as methyl, ethyl, or pivaloyloxymethyl, or acyl derivatives of alcohols, such as acetate or maleate, can be employed. Included are those esters and acyl groups known in the art for modifying the solubility or hydrolysis characteristics for use as sustained-release or prodrug formulations.

Preparation of the Nucleoside Compounds and Derivatives of the Invention

The nucleoside compounds and derivatives thereof of the present invention can be prepared following synthetic methodologies well-established in the practice of nucleoside and nucleotide chemistry. Reference is made to the following text for a description of synthetic methods used in the preparation of the compounds of the present invention: "Chemistry of Nucleosides and Nucleotides," L.B. Townsend, ed., Vols. 1-3, Plenum Press, 1988, which is incorporated by reference herein in its entirety.

The synthesis of 9-(2'-C-methyl-β-D-ribofuranosyl)purines of structural formula VIII is described in U.S. Patent No. 3,480,613, the contents of which are incorporated herein in their entirety.

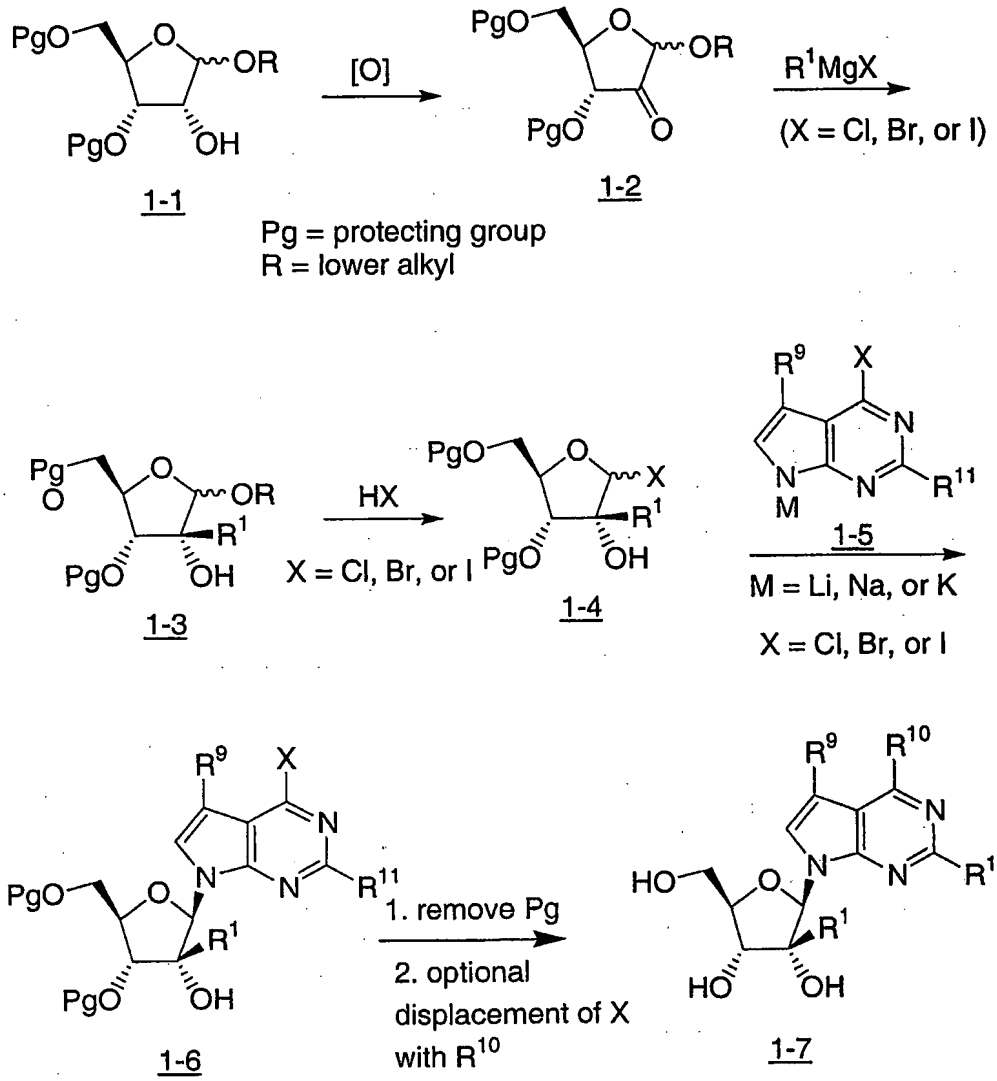


(VIII)

A representative general method for the preparation of compounds of the present invention is outlined in Scheme 1 below. This scheme illustrates the synthesis of compounds of the present invention of structural formula 1-7 wherein the furanose ring has the β-D-ribo configuration. The starting material is a 3,5-bis-O-protected alkyl furanoside, such as methyl furanoside, of structural formula 1-1. The C-2 hydroxyl group is then oxidized with a suitable oxidizing agent, such as a chromium trioxide or chromate reagent, Dess-Martin periodinane, or by Swern oxidation, to afford a C-2 ketone of structural formula 1-2. Addition of a Grignard reagent, such as an alkyl, alkenyl, or alkynyl magnesium halide (for example, MeMgBr, EtMgBr, vinylMgBr, allylMgBr, and ethynylMgBr) or an alkyl, alkenyl, or alkynyl lithium, such as MeLi, across the carbonyl double bond of 1-2 in a suitable organic solvent, such as tetrahydrofuran, diethyl ether, and the like, affords the C-2 tertiary alcohol of structural formula 1-3. A good leaving group (such as Cl, Br, and I) is next introduced at the C-1 (anomeric) position of the furanose sugar derivative by

treatment of the furanoside of formula 1-3 with a hydrogen halide in a suitable organic solvent, such as hydrogen bromide in acetic acid, to afford the intermediate furanosyl halide 1-4. A C-1 sulfonate, such methanesulfonate ($MeSO_2O$-), trifluoromethane-sulfonate (CF_3SO_2O-), or p-toluenesulfonate (-OTs), may also serve as a useful leaving group in the subsequent reaction to generate the glycosidic (nucleosidic) linkage. The nucleosidic linkage is constructed by treatment of the intermediate of structural formula 1-4 with the metal salt (such as lithium, sodium, or potassium) of an appropriately substituted 1*H*-pyrrolo[2,3-d]pyrimidine 1-5, such as an appropriately substituted 4-halo-1*H*-pyrrolo[2,3-d]pyrimidine, which can be generated *in situ* by treatment with an alkali hydride (such as sodium hydride), an alkali hydroxide (such as potassium hydroxide), an alkali carbonate (such as potassium carbonate), or an alkali hexamethyldisilazide (such as NaHMDS) in a suitable anhydrous organic solvent, such as acetonitrile, tetrahydrofuran, 1-methyl-2-pyrrolidinone, or N,N-dimethylformamide (DMF). The displacement reaction can be catalyzed by using a phase-transfer catalyst, such as TDA-1 or triethylbenzyl-ammonium chloride, in a two-phase system (solid-liquid or liquid-liquid). The optional protecting groups in the protected nucleoside of structural formula 1-6 are then cleaved following established deprotection methodologies, such as those described in T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons, 1999. Optional introduction of an amino group at the 4-position of the pyrrolo[2,3-d]pyrimidine nucleus is effected by treatment of the 4-halo intermediate 1-6 with the appropriate amine, such as alcoholic ammonia or liquid ammonia, to generate a primary amine at the C-4 position ($-NH_2$), an alkylamine to generate a secondary amine (-NHR), or a dialkylamine to generate a tertiary amine (-NRR'). A 7H-pyrrolo[2,3-d]pyrimidin-4(3H)one compound may be derived by hydrolysis of 1-6 with aqueous base, such as aqueous sodium hydroxide. Alcoholysis (such as methanolysis) of 1-6 affords a C-4 alkoxide (-OR), whereas treatment with an alkyl mercaptide affords a C-4 alkylthio (-SR) derivative. Subsequent chemical manipulations well-known to practitioners of ordinary skill in the art of organic/medicinal chemistry may be required to attain the desired compounds of the present invention.

Scheme 1

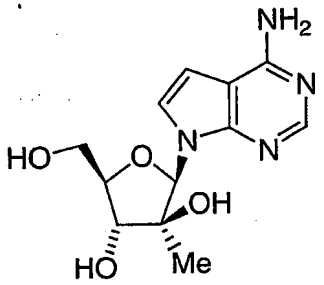


The examples below provide citations to literature publications, which contain details for the preparation of final compounds or intermediates employed in the preparation of final compounds of the present invention. The nucleoside compounds of the present invention were prepared according to procedures detailed in the following examples. The examples are not intended to be limitations on the scope of the instant invention in any way, and they should not be so construed. Those skilled in the art of nucleoside and nucleotide synthesis will readily appreciate that

known variations of the conditions and processes of the following preparative procedures can be used to prepare these and other compounds of the present invention. All temperatures are degrees Celsius unless otherwise noted.

EXAMPLE 1

4-Amino-7-(2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

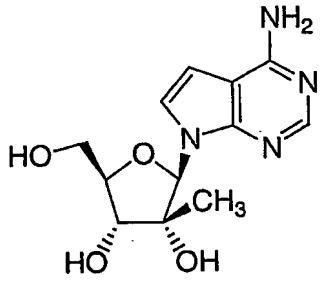


To chromium trioxide (1.57 g, 1.57 mmol) in dichloromethane (DCM) (10 mL) at 0°C was added acetic anhydride (145 mg, 1.41 mmol) and then pyridine (245 mg, 3.10 mmol). The mixture was stirred for 15 min, then a solution of 7-[3,5-*O*-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl]-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine [for preparation, see J. Am. Chem. Soc. 105: 4059 (1983)] (508 mg, 1.00 mmol) in DCM (3 mL) was added. The resulting solution was stirred for 2 h and then poured into ethyl acetate (10 mL), and subsequently filtered through silica gel using ethyl acetate as the eluent. The combined filtrates were evaporated in vacuo, taken up in diethyl ether/THF (1:1) (20 mL), cooled to -78°C and methylmagnesium bromide (3M, in THF) (3.30 mL, 10 mmol) was added dropwise. The mixture was stirred at -78°C for 10 min, then allowed to come to room temperature (rt) and quenched by addition of saturated aqueous ammonium chloride (10 mL) and extracted with DCM (20 mL). The organic phase was evaporated in vacuo and the crude product purified on silica gel using 5% methanol in dichloromethane as eluent. Fractions containing the product were pooled and evaporated *in vacuo*. The resulting oil was taken up in THF (5 mL) and tetrabutylammonium fluoride (TBAF) on silica (1.1 mmol/g on silica) (156 mg) was added. The mixture was stirred at room temperature for 30 min, filtered, and evaporated *in vacuo*. The crude product was purified on silica gel using 10%

methanol in dichloromethane as eluent. Fractions containing the product were pooled and evaporated *in vacuo* to give the desired compound (49 mg) as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 1.08 (s, 3H), 3.67 (m, 2H), 3.74 (m, 1H), 3.83 (m, 1H), 5.19 (m, 1H), 5.23 (m, 1H), 5.48 (m, 1H), 6.08 (1H, s), 6.50 (m, 1H), 6.93 (bs, 2H), 7.33 (m, 1H), 8.02 (s, 1H).

EXAMPLE 2

4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-ribofuranose

A mixture of 2-*O*-acetyl-3,5-bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-ribofuranose [for preparation, see: Helv. Chim. Acta 78: 486 (1995)] (52.4 g, 0.10 mol) in methanolic K_2CO_3 (500 mL, saturated at room temperature) was stirred at room temperature for 45 min and then concentrated under reduced pressure. The oily residue was suspended in CH_2Cl_2 (500 mL), washed with water (300 mL + 5 × 200 mL) and brine (200 mL), dried (Na_2SO_4), filtered, and concentrated to give the title compound (49.0 g) as colorless oil, which was used without further purification in Step B below.

^{1}H NMR (DMSO-d_6): δ 3.28 (s, 3H, OCH_3), 3.53 (d, 2H, $J_{5,4}$ = 4.5 Hz, H-5a, H-5b), 3.72 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,2}$ = 6.6 Hz, H-3), 3.99 (ddd, 1H, $J_{2,1}$ = 4.5 Hz, $J_{2,OH-2}$ = 9.6 Hz, H-2), 4.07 (m, 1H, H-4), 4.50 (s, 2H, CH_2Ph), 4.52, 4.60 (2d, 2H, J_{gem} = 13.6 Hz, CH_2Ph), 4.54 (d, 1H, OH-2), 4.75 (d, 1H, H-1), 7.32-7.45, 7.52-7.57 (2m, 10H, 2Ph).

^{13}C NMR (DMSO-d_6): δ 55.40, 69.05, 69.74, 71.29, 72.02, 78.41, 81.45, 103.44, 127.83, 127.95, 129.05, 129.28, 131.27, 131.30, 133.22, 133.26, 133.55, 133.67, 135.45, 135.92.

Step B: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-*erythro*-pentofuranos-2-ulose

To an ice-cold suspension of Dess-Martin periodinane (50.0 g, 118 mmol) in anhydrous CH_2Cl_2 (350 mL) under argon (Ar) was added a solution of the compound from Step A (36.2 g, 75 mmol) in anhydrous CH_2Cl_2 (200 mL) dropwise over 0.5 h. The reaction mixture was stirred at 0°C for 0.5 h and then at room temperature for 3 days. The mixture was diluted with anhydrous Et_2O (600 mL) and poured into an ice-cold mixture of $Na_2S_2O_3.5H_2O$ (180 g) in saturated aqueous $NaHCO_3$ (1400 mL). The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (600 mL), water (800 mL) and brine (600 mL), dried ($MgSO_4$), filtered and evaporated to give the title compound (34.2 g) as a colorless oil, which was used without further purification in Step C below.

^1H NMR ($CDCl_3$): δ 3.50 (s, 3H, OCH_3), 3.79 (dd, 1H, $J_{5a,5b}$ = 11.3 Hz, $J_{5a,4}$ = 3.5 Hz, H-5a), 3.94 (dd, 1H, $J_{5b,4}$ = 2.3 Hz, H-5b), 4.20 (dd, 1H, $J_{3,1}$ = 1.3 Hz, $J_{3,4}$ = 8.4 Hz, H-3), 4.37 (ddd, 1H, H-4), 4.58, 4.69 (2d, 2H, J_{gem} = 13.0 Hz, CH_2Ph), 4.87 (d, 1H, H-1), 4.78, 5.03 (2d, 2H, J_{gem} = 12.5 Hz, CH_2Ph), 7.19-7.26, 7.31-7.42 (2m, 10H, 2Ph).

^{13}C NMR (DMSO-d_6): δ 55.72, 69.41, 69.81, 69.98, 77.49, 78.00, 98.54, 127.99, 128.06, 129.33, 129.38, 131.36, 131.72, 133.61, 133.63, 133.85, 133.97, 134.72, 135.32, 208.21.

Step C: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose

To a solution of MeMgBr in anhydrous Et_2O (0.48 M, 300 mL) at –55 °C was added dropwise a solution of the compound from Step B (17.40 g, 36.2 mmol) in anhydrous Et_2O (125 mL). The reaction mixture was allowed to warm to –30°C and stirred for 7 h at –30°C to –15°C, then poured into ice-cold water (500 mL) and the mixture vigorously stirred at room temperature for 0.5 h. The mixture was filtered through a Celite pad (10 × 5 cm) which was thoroughly washed with Et_2O. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in hexanes (~30 mL), applied onto a silica gel column (10 × 7 cm, prepacked in hexanes) and eluted with hexanes and hexanes/EtOAc (9/1) to give the title compound (16.7 g) as a colorless syrup.

^{1}H NMR ($CDCl_3$): δ 1.36 (d, 3H, $J_{Me,OH}$ = 0.9 Hz, 2C-Me), 3.33 (q, 1H, OH), 3.41 (d, 1H, $J_{3,4}$ = 3.3 Hz), 3.46 (s, 3H, OCH_3), 3.66 (d, 2H, $J_{5,4}$ = 3.7 Hz, H-5a, H-5b), 4.18 (apparent q, 1H, H-4), 4.52 (s, 1H, H-1), 4.60 (s, 2H, C*H*$_2$Ph), 4.63, 4.81 (2d, 2H, J_{gem} = 13.2 Hz, C*H*$_2$Ph), 7.19-7.26, 7.34-7.43 (2m, 10H, 2Ph).

^{13}C NMR ($CDCl_3$): δ 24.88, 55.45, 69.95, 70.24, 70.88, 77.06, 82.18, 83.01, 107.63, 127.32, 129.36, 130.01, 130.32, 133.68, 133.78, 134.13, 134.18, 134.45, 134.58.

Step D: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step C (9.42 g, 19 mmol) in anhydrous dichloromethane (285 mL) at 0°C was added HBr (5.7 M in acetic acid, 20 mL, 114 mmol) dropwise. The resulting solution was stirred at 0°C for 1 h and then at room temperature for 3 h, evaporated *in vacuo* and co-evaporated with anhydrous toluene (3 × 40 mL). The oily residue was dissolved in anhydrous acetonitrile (50 mL) and added to a solution of the sodium salt of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine in acetonitrile [generated *in situ* from 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see J. Chem. Soc., 131 (1960)] (8.76 g, 57 mmol) in anhydrous acetonitrile (1000 mL), and NaH (60% in mineral oil, 2.28 g, 57 mmol), after 4 h of vigorous stirring at room temperature]. The combined mixture was stirred at room temperature for 24 h, and then evaporated to dryness. The residue was suspended in water (250 mL) and extracted with EtOAc (2 × 500 mL). The combined extracts were washed with brine (300 mL, dried over Na_2SO_4, filtered and evaporated. The crude product was purified on a silica gel column (10 cm × 10 cm) using ethyl acetate/hexane (1:3 and 1:2) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product (5.05 g) as a colorless foam.

^{1}H NMR ($CDCl_3$): δ 0.93 (s, 3H, CH_3), 3.09 (s, 1H, OH), 3.78 (dd, 1H, $J_{5',5''}$ = 10.9 Hz, $J_{5',4}$ = 2.5 Hz, H-5'), 3.99 (dd, 1H, $J_{5'',4}$ = 2.2 Hz, H-5''), 4.23-4.34 (m, 2H, H-3', H-4'), 4.63, 4.70 (2d, 2H, J_{gem} = 12.7 Hz, C*H*$_2$Ph), 4.71, 4.80 (2d, 2H, J_{gem} = 12.1 Hz,C*H*$_2$Ph), 6.54 (d, 1H, , $J_{5,6}$ = 3.8 Hz, H-5), 7.23-7.44 (m, 10H, 2Ph).

^{13}C NMR ($CDCl_3$): δ 21.31, 69.10, 70.41, 70.77, 79.56, 80.41, 81.05, 91.11, 100.57, 118.21, 127.04, 127.46, 127.57, 129.73, 129.77, 130.57, 130.99, 133.51, 133.99, 134.33, 134.38, 134.74, 135.21, 151.07, 151.15 152.47.

Step E: 4-Chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step D (5.42 g, 8.8 mmol) in dichloromethane (175 mL) at -78°C was added boron trichloride (1M in dichloromethane, 88 mL, 88 mmol) dropwise. The mixture was stirred at –78°C for 2.5 h, then at –30°C to –20°C for 3 h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (90 mL) and the resulting mixture stirred at –15°C for 30 min, then neutralized with aqueous ammonia at 0°C and stirred at room temperature for 15 min. The solid was filtered and washed with CH_2Cl_2/MeOH (1/1, 250 mL). The combined filtrate was evaporated, and the residue was purified by flash chromatography over silica gel using CH_2Cl_2 and CH_2Cl_2:MeOH (99:1, 98:2, 95:5 and 90:10) gradient as the eluent to furnish desired compound (1.73 g) as a colorless foam, which turned into an amorphous solid after treatment with MeCN.

^{1}H NMR (DMSO-d_6): δ 0.64 (s, 3H, CH_3), 3.61-3.71 (m, 1H, H-5'), 3.79-3.88 (m, 1H, H-5"), 3.89-4.01 (m, 2H, H-3', H-4'), 5.15-5.23 (m, 3H, 2'-OH, 3'-OH, 5'-OH), 6.24 (s, 1H, H-1'), 6.72 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-5), 8.13 (d, 1H, H-6), 8.65 (s, 1H, H-2).

^{13}C NMR (DMSO-d_6): δ 20.20, 59.95, 72.29, 79.37, 83.16, 91.53, 100.17, 117.63, 128.86, 151.13, 151.19, 151.45.

Step F: 4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step E (1.54 g, 5.1 mmol) was added methanolic ammonia (saturated at 0°C; 150 mL). The mixture was heated in a stainless steel autoclave at 85°C for 14 h, then cooled and evaporated *in vacuo*. The crude mixture was purified on a silica gel column with CH_2Cl_2/MeOH (9/1) as eluent to give the title compound as a colorless foam (0.8 g), which separated as an amorphous solid after treatment with MeCN. The amorphous solid was recrystallized from methanol/acetonitrile; m.p. 222°C.

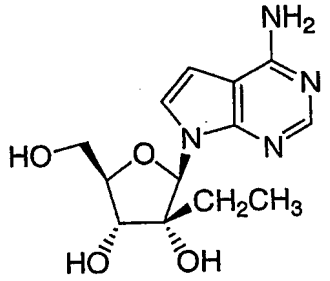
^{1}H NMR (DMSO-d_6): δ 0.62 (s, 3H, CH_3), 3.57-3.67 (m, 1H, H-5'), 3.75-3.97 (m, 3H, H-5", H-4', H-3'), 5.00 (s, 1H, 2'-OH), 5.04 (d, 1H, $J_{3'OH,3'}$ = 6.8 Hz, 3'-OH), 5.06 (t, 1H, $J_{5'OH,5',5"}$ = 5.1 Hz, 5'-OH), 6.11 (s, 1H, H-1'), 6.54 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 6.97 (br s, 2H, NH_2), 7.44 (d, 1H, H-6), 8.02 (s, 1H, H-2).

^{13}C NMR (DMSO-d_6): δ 20.26, 60.42, 72.72, 79.30, 82.75, 91.20, 100.13, 103.08, 121.96, 150.37, 152.33, 158.15.

LC-MS: Found: 279.10 ($M-H^+$); calc. for $C_{12}H_{16}N_4O_4+H^+$: 279.11.

EXAMPLE 3

4-Amino-7-(2-*C*-ethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-ethyl-1-*O*-methyl-α-D-ribofuranose

To diethyl ether (300 mL) at –78°C was slowly added EtMgBr (3.0 M, 16.6 mL) and then dropwise the compound from Step B of Example 2 (4.80 g, 10.0 mmol) in anhydrous Et_2O (100 mL). The reaction mixture was stirred at –78 °C for 15 min, allowed to warm to –15°C and stirred for another 2 h, and then poured into a stirred mixture of water (300 mL) and Et_2O (600 mL). The organic phase was separated, dried ($MgSO_4$), and evaporated *in vacuo*. The crude product was purified on silica gel using ethyl acetate/hexane (1:2) as eluent. Fractions containing the product were pooled and evaporated *in vacuo* to give the desired product (3.87 g) as a colorless oil.

Step B: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-ethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step A (1.02 mg, 2.0 mmol) in dichloromethane (40 mL) was added HBr (5.7 M in acetic acid) (1.75 mL, 10.0 mmol) dropwise at 0°C. The resulting solution was stirred at room temperature for 2 h, evaporated *in vacuo* and co-evaporated twice from toluene (10 mL). The oily residue was dissolved in acetonitrile (10 mL) and added to a vigorously stirred mixture of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine (307 mg, 2.0 mmol), potassium hydroxide (337 mg, 6.0 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (130 mg, 0.4 mmol) in acetonitrile (10 mL). The resulting mixture was stirred at room temperature

overnight, and then poured into a stirred mixture of saturated ammonium chloride (100 mL) and ethyl acetate (100 mL). The organic layer was separated, washed with brine (100 mL), dried over $MgSO_4$, filtered and evaporated *in vacuo*. The crude product was purified on silica gel using ethyl acetate/hexane (1:2) as eluent to give the desired product (307 mg) as a colorless foam.

Step C: 4-Chloro-7-(2-*C*-ethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step B (307 mg, 0.45 mmol) in dichloromethane (8 mL) was added boron trichloride (1M in dichloromethane) (4.50 mL, 4.50 mmol) at -78°C. The mixture was stirred at -78°C for 1h, then at -10°C for 3h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (10 mL), stirred at -15°C for 30 min, and neutralized by addition of aqueous ammonium hydroxide. The mixture was evaporated under diminished pressure and the resulting oil purified on silica gel using methanol/dichloromethane (1:9) as eluent. Fractions containing the product were pooled and evaporated *in vacuo* to give the desired product (112 mg) as a colorless foam.

Step D: 4-Amino-7-(2-*C*-ethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

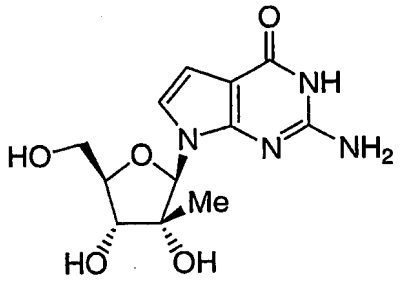
To the compound from Step C (50 mg, 0.16 mmol) was added saturated ammonia in methanol (4 mL). The mixture was stirred at 75°C for 72 h in a closed container, cooled and evaporated *in vacuo*. The crude mixture was purified on silica gel using methanol/dichloromethane (1:9) as eluent. Fractions containing the product were pooled and evaporated *in vacuo* to give the desired product (29 mg) as a colorless powder.

^{1}HNMR (200 MHz, DMSO-d_6): δ 0.52 (t, 3H), 1.02 (m, 2H), 4.01-3.24 (m, 6H), 5.06 (m, 1H), 6.01 (s, 1H), 6.51 (d, 1H), 6.95 (s br, 2H), 6.70 (d, 1H), 7.99 (s, 1H).

LC-MS: Found: 295.2 ($M+H^+$); calc. for $C_{13}H_{18}N_4O_4+H^+$: 295.14.

EXAMPLE 4

2-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one



Step A: 2-Amino-4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold solution of the product from Step C of Example 2 (1.27 g, 2.57 mmol) in CH_2Cl_2 (30 mL) was added HBr (5.7 M in acetic acid; 3 mL) dropwise. The reaction mixture was stirred at room temperature for 2 h, concentrated under diminished pressure and coevaporated with toluene (2 × 15 mL). The resulting oil was dissolved in acetonitrile (MeCN) (15 mL) and added dropwise into a well-stirred mixture of 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see Heterocycles 35: 825 (1993)] (433 mg, 2.57 mmol), KOH (85%, powdered) (0.51 g, 7.7 mmol), tris-[2-(2-methoxyethoxy)ethyl]amine (165 μL, 0.51 mmol) in acetonitrile (30 mL). The resulting mixture was stirred at room temperature for 1h, filtered and evaporated. The residue was purified on a silica gel column using hexanes/EtOAc, 5/1, 3/1 and 2/1, as eluent to give the title compound as a colorless foam (0.65 g).

Step B: 2-Amino-4-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the product from Step A (630 mg, 1.0 mmol) in CH_2Cl_2 (20 mL) at -78°C was added boron trichloride (1M in CH_2Cl_2) (10 mL, 10 mmol). The mixture was stirred at -78°C for 2 h, then at -20°C for 2.5 h. The reaction was quenched with CH_2Cl_2/MeOH (1:1) (10 mL), stirred at -20°C for 0.5 h, and neutralized at 0°C with aqueous ammonia. The solid was filtered, washed with CH_2Cl_2/MeOH (1:1) and the combined filtrate evaporated *in vacuo*. The residue was

purified on a silica gel column with CH_2Cl_2/MeOH, 50/1 and 20/1, as eluent to give the title compound as a colorless foam (250 mg).

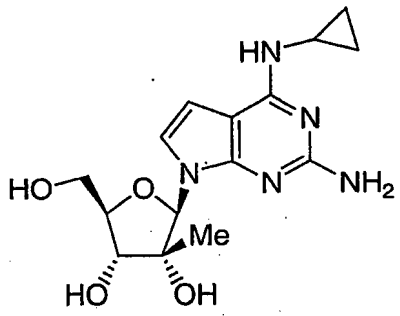
Step C: 2-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one

A mixture of the product from Step B (90 mg, 0.3 mmol) in aqueous NaOH (2N, 9 mL) was heated at reflux temperature for 5 h, then neutralized at 0°C with 2 N aqueous HCl and evaporated to dryness. Purification on a silica gel column with CH_2Cl_2/MeOH, 5/1, as eluent afforded the title compound as a white solid (70 mg).

^{1}H NMR (200 MHz, CD_3OD): δ 0.86 (s, 3H), 3.79 (m 1H), 3.90-4.05 (m, 3H), 6.06 (s, 1H), 6.42 (d, J = 3.7 Hz, 1H), 7.05 (d, J = 3.7 Hz, 1H).

EXAMPLE 5

2-Amino-4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

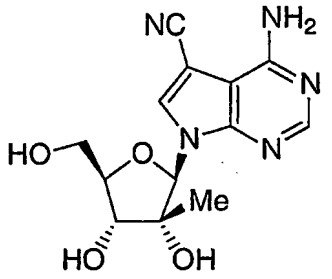


A solution of 2-amino-4-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (Example 4, Step B) (21 mg, 0.07 mmol) in cyclopropylamine (0.5 mL) was heated at 70°C for two days, then evaporated to an oily residue and purified on a silica gel column with CH_2Cl_2/MeOH, 20/1, as eluent to give the title compound as a white solid (17 mg).

^{1}H NMR (200 MHz, CD_3CN): δ 0.61 (m, 2H), 0.81 (m, 2H), 0.85 (s, 3H), 2.83 (m, 1H), 3.74-3.86 (m, 1H), 3.93-4.03 (m, 2H), 4.11 (d, J = 8.9 Hz, 1H), 6.02 (s, 1H), 6.49 (d, J = 3.7 Hz, 1H), 7.00 (d, J = 3.7 Hz, 1H).

EXAMPLE 6

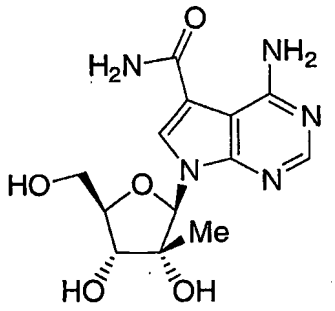
4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile



This compound was prepared following procedures described by Y. Murai et al. in Heterocycles 33: 391-404 (1992).

EXAMPLE 7

4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide



This compound was prepared following procedures described by Y. Murai et al. in Heterocycles 33: 391-404 (1992).

EXAMPLE 8

General process to SATE prodrug moiety

S-Acyl-2-thioethyl (SATE) pronucleotides are discussed in C.R. Wagner, V.V. Iyer, and E.J. McIntee, "Pronucleotides: Toward the In Vivo Delivery of Antiviral and Anticancer Nucleotides," Med. Res. Rev., 20: 1-35 (2000), which is

incorporated by reference herein in its entirety. SATE derivatives of nucleosides are also disclosed U.S. Patent Nos. 5,770,725; 5,849,905; and 6,020,482, the contents of each of which are incorporated by reference herein in their entirety.

Bis(*S*-acetyl-2-thioethyl)-*N*,*N*-diisopropylphosphoramidite

2-Mercaptoethanol (5 g, 64 mmol) was dissolved in CH_2Cl_2 (50 mL). To this solution was added triethylamine (7.67 mL, 57.6 mmol), and the reaction mixture was cooled in an ice bath to 0 °C. Acetic anhydride (4.54 mL, 48 mmol) was added dropwise in 10 min, and the reaction mixture was stirred for 1 h at 0 °C. The reaction mixture was then allowed to come to room temperature over a period of 2 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with water (75 mL), 5% aqueous $NaHCO_3$ (75 mL) and brine (75 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oil. The oil was then dissolved in anhydrous THF (40 mL) and anhydrous triethylamine (7.76 mL) was added. To this mixture was added activated molecular sieves (4Å) and was kept at room temperature for 10 min. The reaction mixture was cooled in an ice bath to 0°C and diisopropylphosphoramidous dichloride (6.47 g, 32.03 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h under inert atmosphere. Hexane (40 mL) was added to the reaction mixture and the precipitate formed was filtered. The filtrate was concentrated to one fourth of the volume, purified by loaded silica gel column chromatography and eluted with hexane containing 3 % triethylamine and incremental amount of ethyl acetate (0 to 7 %) to give the title compound as an oil (2.36 g). ^{1}H NMR ($CDCl_3$): δ 1.17 (s, 6H), 1.21 (s, 6H), 2.36 (s, 6H), 3.14 (t, J = 6.44 Hz), 3.51-3.84 (m, 6H); ^{13}C NMR ($CDCl_3$): δ 24.47, 24.61, 30.48, 42.85, 43.1, 61.88, 62.23, 195.26; ^{13}P NMR ($CDCl_3$): δ 146.96.

EXAMPLE 9

5'-Triphosphate Derivatives

The nucleoside 5'-triphosphates of the present invention were prepared following general procedures described in *Chem. Rev.* 100: 2047 (2000).

EXAMPLE 10

Purification and Purity Analysis of 5'-Triphosphate Derivatives

The triphosphate derivatives were purified by anion exchange (AX) chromatography using a 30 x 100 mm Mono Q column (Pharmacia) with a buffer system of 50 mM Tris, pH 8. Elution gradients were typically from 40 mM NaCl to 0.8 M NaCl in two column volumes at 6.5 mL/min. Appropriate fractions from anion exchange chromatography were collected and desalted by reverse-phase (RP) chromatography using a Luna C18 250 × 21 mm column (Phenomenex) with a flow rate of 10 mL/min. Elution gradients were generally from 1% to 95% methanol in 14 min at a constant concentration of 5 mM triethylammonium acetate (TEAA).

Mass spectra of the purified triphosphates were determined using on-line HPLC mass spectrometry on a Hewlett-Packard (Palo Alto, CA) MSD 1100. A Phenomenex Luna (C18(2)), 150 × 2 mm, plus 30 x 2 mm guard column, 3-μm particle size was used for RP HPLC. A 0 to 50% linear gradient (15 min) of acetonitrile in 20 mM TEAA (triethylammonium acetate) pH 7 was performed in series with mass spectral detection in the negative ionization mode. Nitrogen gas and a pneumatic nebulizer were used to generate the electrospray. The mass range of 150-900 was sampled. Molecular masses were determined using the HP Chemstation analysis package.

The purity of the purified triphosphates was determined by analytical RP and AX HPLC. RP HPLC with a Phenomonex Luna or Jupiter column (250 × 4.6 mm), 5-μm particle size was typically run with a 2-70% acetonitrile gradient in 15 min in 100 mM TEAA, pH 7. AX HPLC was performed on a 1.6 × 5 mm Mono Q column (Pharmacia). Triphosphates were eluted with a gradient of 0 to 0.4 M NaCl at constant concentration of 50 mM Tris, pH 8. The purity of the triphosphates was generally >80%.

EXAMPLE 11

5'-Monophosphate Derivatives

The nucleoside 5'-monophosphates of the present invention were prepared following the general procedures described in *Tetrahedron Lett.* 50: 5065 (1967).

EXAMPLE 12

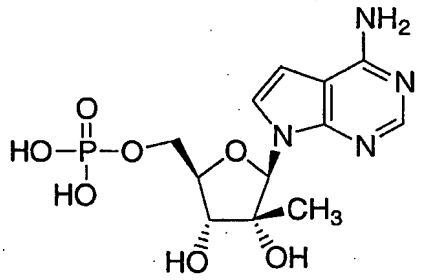
Mass Spectral Characterization of 5'-Triphosphate Derivatives

Mass spectra of 5'-triphosphates of the compounds of the present invention were determined as described in Example 10. Listed in the following table are the calculated and experimental masses for representative 5'-triphosphates prepared according to the procedures of Example 9. The example numbers correspond to the parent compound of the 5'-triphosphate.

Example	Calculated	Found
1	520.0	519.9
2	520.0	520.0
3	534.0	534.0
4	536.0	536.0

EXAMPLE 13

[4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine]-5'-monophosphate

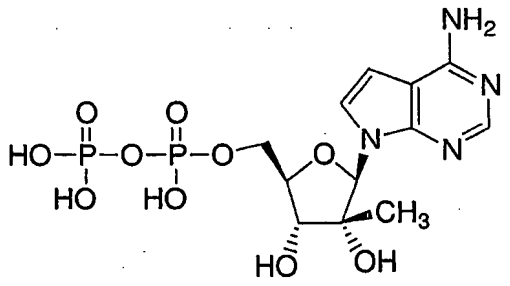


To the compound from Step F of Example 2 (14 mg, 0.05 mmol) (dried by coevaporation with pyridine and several times with toluene) was added trimethyl phosphate (0.5 mL). The mixture was stirred overnight in a sealed container. It was then cooled to 0°C and phosphorous oxychloride (0.0070 mL, 0.075 mmol) was added via a syringe. The mixture was stirred for 3 h at 0°C, then the reaction was quenched by addition of tetraethylammonium bicarbonate (TEAB) (1M)

(0.5 mL) and water (5 mL). The reaction mixture was purified and analyzed according to the procedure described in Example 10.
Electron spray mass spectrum (ES-MS): Found: 359.2 ($M-H^+$), calc. for $C_{12}H_{17}N_4O_7P - H^+$: 359.1.

EXAMPLE 14

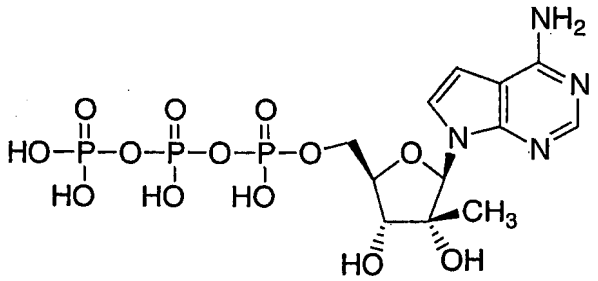
[4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine]-5'-diphosphate



To the compound from Step F of Example 2 (56 mg, 0.20 mmol) (dried by coevaporation with pyridine and several times with toluene) was added trimethyl phosphate (stored over sieves) (1.0 mL). The mixture was stirred overnight in a sealed container. It was then cooled to 0°C and phosphorous oxychloride (0.023 mL, 0.25 mmol) was added via a syringe. The mixture was stirred for 2 h at 0°C, then tributylamine (0.238 mL, 1.00 mmol) and tributylammonium phosphate (generated from phosphoric acid and tributylamine in pyridine, followed by repeated azeotropic evaporation with pyridine and acetonitrile) (1.0 mmol in 3.30 mL acetonitrile) was added. The mixture was stirred for an additional 30 min at 0°C, the sealed vial was then opened and the reaction quenched by addition of TEAB (1M) (1.0 mL) and water (5 mL). The reaction mixture was purified and analyzed according to the procedure described in Example 10.
ES-MS: Found: 439.0 ($M-H^+$), calc. for $C_{12}H_{18}N_4O_{10}P_2 - H^+$: 439.04.

EXAMPLE 15

[4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine]-5'-triphosphate

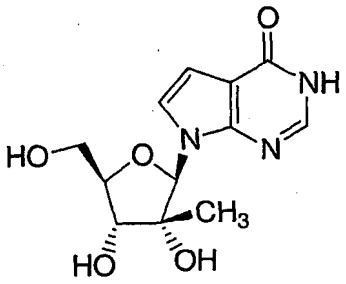


To the compound from Step F of Example 2 (20 mg, 0.07 mmol) (dried by coevaporation with pyridine and several times with toluene) was added trimethyl phosphate (stored over sieves) (0.4 mL). The mixture was stirred overnight in a sealed container. It was then cooled to 0°C and phosphorous oxychloride (0.0070 mL, 0.075 mmol) was added via syringe. The mixture was stirred for 3 h at 0°C, then tributylamine (0.083 mL, 0.35 mmol), tributylammonium pyrophosphate (127 mg, 0.35 mmol) and acetonitrile (stored over sieves) (0.25 mL) were added. The mixture was stirred for an additional 30 min at 0°C, the sealed vial was then opened and the reaction quenched by addition of TEAB (1M) (0.5 mL) and water (5 mL). The reaction mixture was purified and analyzed according to the procedure described in Example 10.

ES-MS: Found: 519.0 ($M-H^+$), calc. for $C_{12}H_{19}N_4O_{13}P_3$- H^+: 519.01.

EXAMPLE 16

7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one

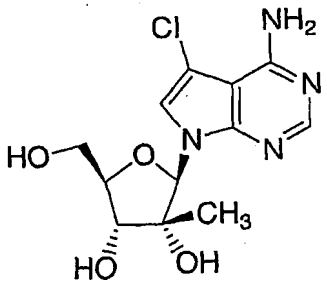


To the compound from Step E of Example 2 (59 mg, 0.18 mmol) was added aqueous sodium hydroxide (1M). The mixture was heated to reflux for 1hr, cooled, neutralized with aqueous HCl (2M) and evaporated in vacuo. The residue was purified on silica gel using dichloromethane/methanol (4:1) as eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (53 mg) as a colorless oil.

^{1}H NMR (CD_3CN): δ 0.70 (s, 3H), 3.34-4.15 (overlapping m, 7H), 6.16 (s, 1H), 6.57 (d, 3.6 Hz, 1H), 7.37 (d, 3.6 Hz, 1H), 8.83 (s, 1H).

EXAMPLE 17

4-Amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



To a pre-cooled solution (0°C) of the compound from Step F of Example 2 (140 mg, 0.50 mmol) in DMF (2.5 mL) was added *N*-chlorosuccinimide (0.075 g, 0.55 mmol) in DMF (0.5 mL) dropwise. The solution was stirred at room

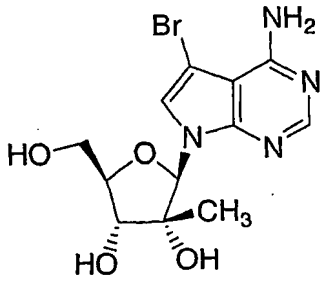
temperature for 1h and the reaction quenched by addition of methanol (4 mL) and evaporated in vacuo. The crude product was purified on silica gel using methanol/dichloromethane (1:9) as eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (55 mg) as a colorless solid.

^{1}H NMR (CD_3CN): δ 0.80 (s, 3H), 3.65-4.14 (overlapping m, 7H), 5.97 (s br, 2H), 6.17 (s, 1H), 7.51 (s, 1H), 8.16 (s, 1H).

ES-MS: Found: 315.0 ($M+H^+$), calc.for $C_{12}H_{15}ClN_4O_4 + H^+$: 315.09.

EXAMPLE 18

4-Amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



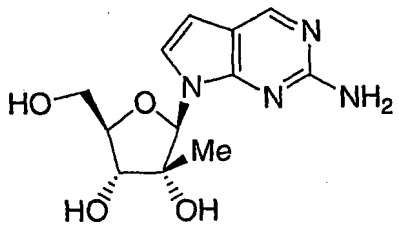
To a pre-cooled solution (0°C) of the compound from Step F of Example 2 (28 mg, 0.10 mmol) in DMF (0.5 mL) was added *N*-bromosuccinimide (0.018 g, 0.10 mmol) in DMF (0.5 mL) dropwise. The solution was stirred at 0°C for 20 min, then at room temperature for 10 min. The reaction was quenched by addition of methanol (4 mL) and evaporated in vacuo. The crude product was purified on silica gel using methanol/dichloromethane (1:9) as eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (13.0 mg) as a colorless solid.

^{1}H NMR (CD_3CN): δ 0.69 (s, 3H), 3.46-4.00 (overlapping m, 7H), 5.83 (s br, 2H), 6.06 (s, 1H), 7.45 (s, 1H), 8.05 (s, 1H).

ES-MS: Found: 359.1 ($M+H^+$), calc.for $C_{12}H_{15}BrN_4O_4 + H^+$: 359.04.

EXAMPLE 19

2-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

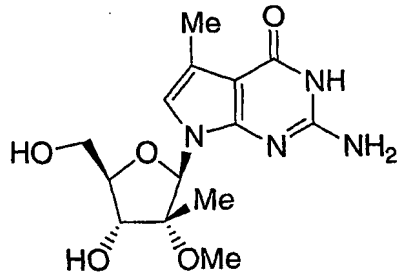


A mixture of 2-amino-4-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (Example 4, Step B) (20 mg, 0.07 mmol) in EtOH (1.0 mL), pyridine (0.1 mL) and 10% Pd/C (6 mg) under H_2 (atmospheric pressure) was stirred overnight at room temperature. The mixture was filtered through a Celite pad which was thorougly washed with EtOH. The combined filtrate was evaporated and purified on a silica gel column with CH_2Cl_2/MeOH, 20/1 and 10/1, as eluent to give the title compound as a white solid (16 mg).

^{1}H NMR (200 MHz, CD_3OD): δ 0.86 (s, 3H, 2'C-Me), 3.82 (dd, $J_{5',4'}$ = 3.6 Hz, $J_{5',5''}$ = 12.7 Hz, 1H, H-5'), 3.94-4.03 (m, 2H, H-5', H-4'), 4.10 (d, $J_{3',4'}$ = 8.8 Hz, 1H, H-3'), 6.02 (s, 1H, H-1'), 6.41 (d, $J_{5,6}$ = 3.8 Hz, 1H, H-5), 7.39 (d, 1H, H-6), 8.43 (s, 1H, H-4). ES MS: 281.4 (MH^+).

EXAMPLE 20

2-Amino-5-methyl-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one



Step A: 2-Amino-4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold solution of the product from Step C of Example 2 (1.57 g, 3.16 mmol) in CH_2Cl_2 (50 mL) was added HBr (5.7 M in acetic acid; 3.3 mL) dropwise. The reaction mixture was stirred at 0°C for 1 h and then at room temperature for 2 h, concentrated in vacuo and co-evaporated with toluene (2 × 20 mL). The resulting oil was dissolved in MeCN (20 mL) and added dropwise to a solution of the sodium salt of 2-amino-4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine in acetonitrile [generated *in situ* from 2-amino-4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see Liebigs Ann. Chem. 1984: 708-721] (1.13 g, 6.2 mmol) in anhydrous acetonitrile (150 mL), and NaH (60% in mineral oil, 248 mg, 6.2 mmol), after 2 h of vigorous stirring at room temperature]. The combined mixture was stirred at room temperature for 24 h and then evaporated to dryness. The residue was suspended in water (100 mL) and extracted with EtOAc (300 + 150 mL). The combined extracts were washed with brine (100 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified on a silica gel column (5 × 7 cm) using ethyl acetate/hexane (0 to 30% EtOAc in 5% step gradient) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product (0.96 g) as a colorless foam.

Step B: 2-Amino-4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold mixture of the product from Step A (475 mg, 0.7 mmol) in THF (7 mL) was added NaH (60% in mineral oil, 29 mg) and stirred at 0 °C for 0.5 h. Then MeI (48 µL) was added and reaction mixture stirred at room temperature for 24 h. The reaction was quenched with MeOH and the mixture evaporated. The crude product was purified on a silica gel column (5 × 3.5 cm) using hexane/ethyl acetate (9/1, 7/1, 5/1 and 3/1) as eluent. Fractions containing the product were combined and evaporated to give the desired compound (200 mg) as a colorless foam.

Step C: 2-Amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-4(3*H*)-one

A mixture of the product from Step B (200 mg, 0.3 mmol) in 1,4-dioxane (15 mL) and aqueous NaOH (2N, 15 mL) in a pressure bottle was heated

overnight at 135 °C. The mixture was then cooled to 0 °C, neutralized with 2N aqueous HCl and evaporated to dryness. The crude product was suspended in MeOH, filtered, and the solid thoroughly washed with MeOH. The combined filtrate was concentrated, and the residue purified on a silica gel column (5 × 5 cm) using CH_2Cl_2/MeOH (40/1, 30/1 and 20/1) as eluent to give the desired compound (150 mg) as a colorless foam.

Step D: 2-Amino-5-methyl-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one

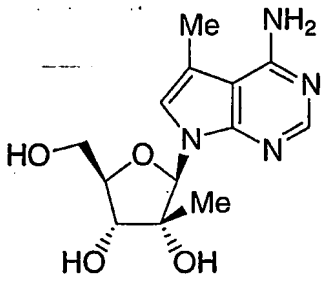
A mixture of the product from Step C (64 mg, 0.1 mmol) in MeOH (5 mL) and Et_3N (0.2 mL) and 10% Pd/C (24 mg) was hydrogenated on a Parr hydrogenator at 50 psi at r.t. for 1.5 days, then filtered through a Celite pad which was thoroughly washed with MeOH. The combined filtrate was evaporated and the residue purified on a silica gel column (3 × 4 cm) with CH_2Cl_2/MeOH (30/1, 20/1) as eluent to yield 2-amino-5-methyl-7-(5-*O*-benzyl-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one. The compound (37 mg) was further hydrogenated in EtOH (2 mL) with 10% Pd/C and under atmospheric pressure of hydrogen. After stirring 2 days at r.t., the reaction mixture was filtered through Celite, the filtrate evaporated and the crude product purified on a silica gel column (1 × 7 cm) with CH_2Cl_2/MeOH (30/1, 20/1 and 10/1) as eluent to yield the title compound (12 mg) after freeze-drying.

^{1}H NMR (200 MHz, CD_3OD): δ 0.81 (s, 3H, 2'C-Me), 2.16 (d, $J_{H\text{-}6,C5\text{-}Me}$ = 1.3 Hz, 3H, C5-Me), 3.41 (s, 3H, 2'-OMe), 3.67 (dd, $J_{5',4'}$= 3.4 Hz, $J_{5',5''}$ = 12.6 Hz, 1H, H-5'), 3.81-3.91 (m, 3H, H-5", H-4', H-3'), 6.10 (s, 1H, H-1'), 6.66 (d, 1H, H-6).

ES MS: 323.3 $(M\text{-}H)^+$.

EXAMPLE 21

4-Amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold solution of the product from Step C of Example 2 (1.06 g, 2.1 mmol) in CH_2Cl_2 (30 mL) was added HBr (5.7 M in acetic acid; 2.2 mL) dropwise. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 2 h, concentrated in vacuo and co-evaporated with toluene (2 × 15 mL). The resulting oil was dissolved in MeCN (10 mL) and added dropwise into a solution of the sodium salt of 4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine in acetonitrile [generated *in situ* from 4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see J. Med. Chem. 33: 1984 (1990)] (0.62 g, 3.7 mmol) in anhydrous acetonitrile (70 mL), and NaH (60% in mineral oil, 148 mg, 3.7 mmol), after 2 h of vigorous stirring at room temperature]. The combined mixture was stirred at room temperature for 24 h and then evaporated to dryness. The residue was suspended in water (100 mL) and extracted with EtOAc (250 + 100 mL). The combined extracts were washed with brine (50 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified on a silica gel column (5 × 5 cm) using hexane/ethyl acetate (9/1, 5/1, 3/1) gradient as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product (0.87 g) as a colorless foam.

Step B: 4-Chloro-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

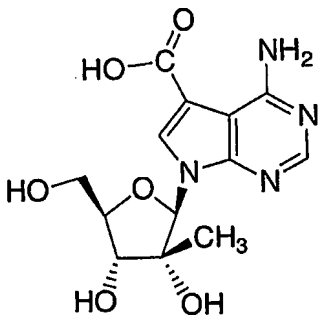
To a solution of the compound from Step A (0.87 g, 0.9 mmol) in dichloromethane (30 mL) at -78°C was added boron trichloride (1M in dichloromethane, 9.0 mL, 9.0 mmol) dropwise. The mixture was stirred at –78°C for 2.5 h, then at –30°C to –20°C for 3 h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (9 mL) and the resulting mixture stirred at –15°C for 30 min, then neutralized with aqueous ammonia at 0°C and stirred at room temperature for 15 min. The solid was filtered and washed with CH_2Cl_2/MeOH (1/1, 50 mL). The combined filtrate was evaporated, and the residue was purified on a silica gel column (5 × 5 cm) using CH_2Cl_2 and CH_2Cl_2/MeOH (40/1 and 30/1) gradient as the eluent to furnish the desired compound (0.22 g) as a colorless foam.

Step C: 4-Amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step B (0.2 g, 0.64 mmol) was added methanolic ammonia (saturated at 0°C; 40 mL). The mixture was heated in a stainless steel autoclave at 100°C for 14 h, then cooled and evaporated *in vacuo*. The crude mixture was purified on a silica gel column (5 × 5 cm) with CH_2Cl_2/MeOH (50/1, 30/1, 20/1) gradient as eluent to give the title compound as a white solid (0.12 g). ^1H NMR (DMSO-d_6): δ 0.60 (s, 3H, 2'C-Me), 2.26 (s, 3H, 5C-Me), 3.52-3.61 (m, 1H, H-5'), 3.70-3.88 (m, 3H, H-5", H-4', H-3'), 5.00 (s, 1H, 2'-OH), 4.91-4.99 (m, 3H, 2'-OH, 3'-OH, 5'-OH), 6.04 (s, 1H, H-1'), 6.48 (br s, 2H, NH_2), 7.12 (s, 1H, H-6), 7.94 (s, 1H, H-2). ES MS: 295.2 (MH^+).

EXAMPLE 22

4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid

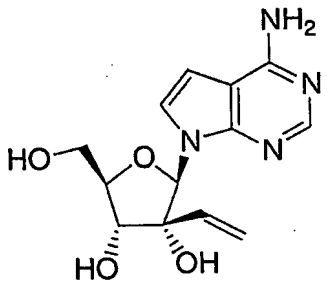


The compound of Example 6 (0.035 g, 0.11 mmol) was dissolved in a mixture of aqueous ammonia (4 mL, 30 wt %) and saturated methanolic ammonia (2 mL), and a solution of H_2O_2 in water (2 mL, 35 wt %) was added. The reaction mixture was stirred at room temperature for 18 h. Solvent was removed under reduced pressure, and the residue obtained was purified by HPLC on a reverse phase column (Altech Altima C-18, 10x 299 mm, A = water, B = acetonitrile, 10 to 60 % B in 50 min, flow 2 mL/min) to yield the title compound (0.015 g, 41 %) as a white solid.

^{1}H NMR (CD_3OD): δ 0.85 (s, 3H, Me), 3.61 (m, 1H), 3.82 (m, 1H) 3.99-4.86 (m, 2H), 6.26 (s, 1H), 8.10 (s, 2H) 8.22(s, 1H); ^{13}C NMR (CD_3OD): 20.13, 61.37, 73.79, 80.42, 84.01, 93.00, 102.66, 112.07, 130.07, 151.40, 152.74, 159.12, 169.30. HRMS (FAB) Calcd for $C_{13}H_{17}N_4O_6^+$ 325.1148, found 325.1143.

EXAMPLE 23

4-Amino-7-(2-*C*-vinyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-vinyl-1-*O*-methyl-α-D-ribofuranose

Cerium chloride heptahydrate (50 g, 134.2 mmol) was finely crushed in a pre-heated mortar and transferred to a round-bottom flask equipped with a mechanical stirrer. The flask was heated under high vacuum overnight at 160°C. The vacuum was released under argon and the flask was cooled to room temperature. Anhydrous THF (300 mL) was cannulated into the flask. The resulting suspension was stirred at room temperature for 4 h and then cooled to –78 °C. Vinylmagnesium bromide (1M in THF, 120 mL, 120 mmol) was added and stirring continued at –78 °C for 2 h. To this suspension was added a solution of 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-erythro-pentofuranose-2-ulose (14 g, 30 mmol) [from Example 2, Step B] in anhydrous THF (100 mL), dropwise with constant stirring. The reaction was stirred at –78 °C for 4 h. The reaction was quenched with saturated ammonium chloride solution and allowed to come to room temperature. The mixture was filtered through a celite pad and the residue washed with Et_2O (2 × 500 mL). The organic layer was separated and the aqueous layer extracted with Et_2O (2 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to a viscous yellow oil. The oil was purified by flash chromatography (SiO_2, 10% EtOAc in hexanes). The title compound (6.7 g, 13.2 mmol) was obtained as a pale yellow oil.

Step B: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-vinyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step A (6.4 g, 12.6 mmol) in anhydrous dichloromethane (150 mL) at –20 °C was added HBr (30% solution in AcOH, 20 mL, 75.6 mmol) dropwise. The resulting solution was stirred between –10°C and 0°C for 4 h, evaporated in vacuo and co-evaporated with anhydrous toluene (3 × 40 mL). The oily residue was dissolved in anhydrous acetonitrile (100 mL) and added to a solution of the sodium salt of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine (5.8 g, 37.8 mmol) in acetonitrile (generated in situ as described in Example 2) at –20 °C. The resulting mixture was allowed to come to room temperature and stirred at room temperature for 24 h. The mixture was then evaporated to dryness, taken up in water and extracted with EtOAc (2 × 300 mL). The combined extracts were dried over Na_2SO_4, filtered and evaporated. The crude mixture was purified by flash chromatography (SiO_2, 10% EtOAc in hexanes) and the title compound (1.75 g) isolated as a white foam.

Step C: 4-Amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-vinyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step B (80, mg) was dissolved in the minimum amount of 1,4-dioxane and placed in a stainless steel bomb. The bomb was cooled to –78°C and liquid ammonia was added. The bomb was sealed and heated at 90°C for 24 h. The ammonia was allowed to evaporate and the residue concentrated to a white solid which was used in the next step without further purification.

Step D: 4-Amino-7-(2-*C*-vinyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

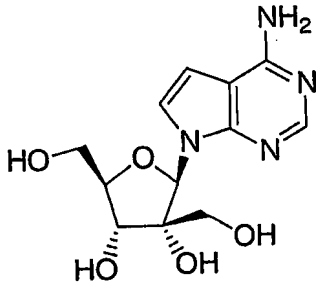
To a solution of the compound from Step C (60 mg) in dichloromethane at –78 °C was added boron trichloride (1M in dichloromethane) dropwise. The mixture was stirred at –78 °C for 2.5 h, then at –30 °C to –20 °C for 3 h. The reaction was quenched by addition of methanol/dichloromethane (1:1) and the resulting mixture stirred at –15 °C for 0.5 h, then neutralized with aqueous ammonia at 0°C and stirred at room temperature for 15 min. The solid was filtered and washed with methanol/dichloromethane (1:1). The combined filtrate was evaporated and the residue purified by flash chromatography (SiO_2, 10% methanol in EtOAc containing 0.1% triethylamine). The fractions containing the product were evaporated to give the title compound as a white solid (10 mg).

^1H NMR (DMSO-d_6): δ 3.6 (m, 1H, H-5'), 3.8 (m, 1H, H-5"), 3.9 (m d, 1-H, H-4'), 4.3 (t, 1H, H-3'), 4.8-5.3(m, 6H, CH=CH_2, 2'-OH, 3'-OH, 5'-OH) 6.12 (s, 1H, H-1'), 6.59 (d, 1H, H-5), 7.1 (br s, 1H, NH2), 7.43 (d, 1H, H-6), 8.01 (s, 1H, H-2).

ES-MS: Found: 291.1 ($M-H^-$); calc. for $C_{13}H_{16}N_4O_4$ - H^-: 291.2.

EXAMPLE 24

4-Amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-hydroxymethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Example 23, Step B (300 mg, 0.48 mmol) in 1,4-dioxane (5 mL) were added *N*-methylmorpholine-*N*-oxide (300 mg, 2.56 mmol) and osmium tetroxide (4% solution in water, 0.3 mL). The mixture was stirred in the dark for 14 h. The precipitate was removed by filtration through a celite plug, diluted with water (3 ×), and extracted with EtOAc. The EtOAc layer was dried over Na_2SO_4 and concentrated in vacuo. The oily residue was taken up in dichloromethane (5 mL) and stirred over $NaIO_4$ on silica gel (3 g, 10% $NaIO_4$) for 12 h. The silica gel was removed by filtration and the residue was evaporated and taken up in absolute ethanol (5 mL). The solution was cooled in an ice bath and sodium borohydride (300 mg, 8 mmol) was added in small portions. The resulting mixture was stirred at room temperature for 4 h and then diluted with EtOAc. The organic layer was washed with water (2 × 20 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was evaporated and the residue purified by flash chromatography (SiO_2, 2:1 hexanes/EtOAc) to give the title compound (160 mg, 0.25 mmol) as white flakes.

Step B: 4-Amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-hydroxymethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step A (150 mg, 0.23 mmol) was dissolved in the minimum amount of 1,4-dioxane (10 mL) and placed in a stainless steel bomb. The bomb was cooled to –78 °C and liquid ammonia was added. The bomb was sealed and heated at 90°C for 24 h. The ammonia was allowed to evaporate and the residue concentrated to a white solid which was used in the next step without further purification.

Step C: 4-Amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

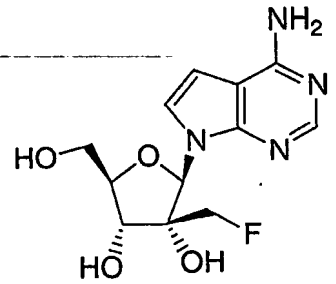
The compound from Step B (120 mg, 0.2 mmol) was dissolved in 1:1 methanol/dichloromethane, 10% Pd-C was added, and the suspension stirred under an H_2 atmosphere for 12 h. The catalyst was removed by filtration through a celite pad and washed with copious amounts of methanol. The combined filtrate was evaporated in vacuo and the residue was purified by flash chromatography (SiO_2, 10% methanol in EtOAc containing 0.1% triethylamine) to give the title compound (50 mg) as a white powder.

^1H NMR (CD_3OD): δ 3.12 (d, 1H, CH_2'), 3.33 (d, 1H, CH_2''), 3.82 (m, 1H, H-5'), 3.99-4.1(m, 2H, H-4', H-5"), 4.3 (d, 1H, H-3'), 6.2 (s, 1H, H-1'), 6.58 (d, 1H, H-5), 7.45 (d, 1H, H-6), 8.05 (s, 1H, H-2).

LC-MS: Found: 297.2 ($M+H^+$); calc. for $C_{12}H_{16}N_4O_5 + H^+$: 297.3.

EXAMPLE 25

4-Amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-fluoromethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Example 24, Step A (63 mg, 0.1 mmol) in anhydrous dichloromethane (5 mL) under argon, were added 4-dimethylaminopyridine (DMAP) (2 mg, 0.015 mmol) and triethylamine (62 μL, 0.45 mmol). The solution was cooled in an ice bath and p-toluenesulfonyl chloride (30 mg, 0.15 mmol) was added. The reaction was stirred at room temperature overnight, washed with $NaHCO_3$ (2 × 10 mL), water (10 mL), brine (10 mL), dried over Na_2SO_4 and concentrated to a pink solid in vacuo. The solid was dissolved in anhydrous THF (5 mL) and cooled in an icebath. Tetrabutylammonium fluoride (1M solution in THF, 1 mL, 1 mmol) was added and the mixture stirred at room temperature for 4 h. The solvent was removed in vacuo, the residue taken up in dichloromethane, and washed with $NaHCO_3$ (2 × 10 mL), water (10 mL) and brine (10 mL). The dichloromethane layer was dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by flash chromatography (SiO_2, 2:1 hexanes/EtOAc) to afford the title compound (20 mg) as a white solid.

Step B: 4-Amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-fluoromethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step A (18 mg, 0.03 mmol) was dissolved in the minimum amount of 1,4-dioxane and placed in a stainless steel bomb. The bomb was cooled to –78 °C and liquid ammonia was added. The bomb was sealed and heated at

90 °C for 24 h. The ammonia was allowed to evaporate and the residue concentrated to a white solid which was used in the next step without further purification.

Step C: 4-Amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step B (16 mg) was dissolved in 1:1 methanol/dichloromethane, 10% Pd-C was added, and the suspension stirred under an H_2 atmosphere for 12 h. The catalyst was removed by filtration through a celite pad and washed with copious amounts of methanol. The combined filtrate was evaporated in vacuo and the residue was purified by flash chromatography (SiO_2, 10% methanol in EtOAc containing 0.1% triethylamine) to give the title compound (8 mg) as a white powder.

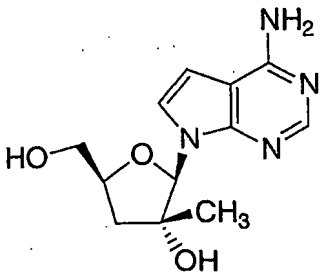
^1H NMR (DMSO-d_6): δ 3.6-3.7 (m, 1H, H-5'), 3.8 – 4.3 (m, 5H, H-5'', H-4', H-3', CH_2) 5.12 (t, 1H, 5'-OH), 5.35 (d, 1H, 3'-OH), 5.48 (s, 1H, 2'-OH), 6.21 (s, 1H, H-1'), 6.52 (d, 1H, H-5), 6.98 (br s, 2H, NH2), 7.44 (d, 1 H, H-6), 8.02 (s, 1H, H-2).

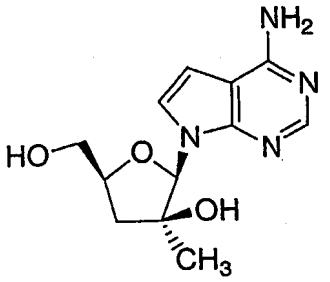
^{19}F NMR (DMSO-d_6): δ -230.2 (t).

ES-MS: Found: 299.1 ($M+H^+$), calc.for $C_{12}H_{15}FN_4O_4 + H^+$: 299.27.

EXAMPLES 26 and 27

4-Amino-7-(3-deoxy-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine and 4-amino-7-(3-deoxy-2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine





Step A: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine and 7-[3,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*] pyrimidine

To a stirred solution of tubercidin (5.0 g, 18.7 mmol) in a mixture of pyridine (7.5 mL) and DMF (18.5 mL) was added silver nitrate (6.36 g, 38.8 mmol). This mixture was stirred at room temperature for 2 h. It was cooled in an ice bath and THF (37.4 mL) and *tert*-butyldimethylsilyl chloride (5.6 g, 37 mmol) was added and the mixture was stirred at room temperature for 2 h. The mixture was then filtered through a pad of celite and washed with THF. The filtrate and washings were diluted with ether containing a small amount of chloroform. The organic layer was washed successively with sodium bicarbonate and water (3 × 50 mL), dried over anhydrous sodium sulfate and concentrated. The pyridine was removed by coevaporation with toluene and the residue was purified by flash chromatography on silica gel using 5-7% MeOH in CH_2Cl_2 as the eluent; yield 3.0 g.

Step B: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)]-4-[di-(4-methoxyphenyl)phenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine and 7-[3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-[di-(4-methoxyphenyl)phenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of mixture of the compounds from Step A (3.0 g, 6.0 mmol) in anhydrous pyridine (30 mL) was added 4,4'-dimethoxytrityl chloride (2.8 g, 8.2 mmol) and the reaction mixture was stirred at room temperature overnight. The mixture was then triturated with aqueous pyridine and extracted with ether. The organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated to a yellow foam (5.6 g). The residue was purified by flash chromatography over silica gel using 20-25% EtOAc in hexanes as the eluent. The appropriate fractions were collected and concentrated to furnish 2',5'-bis-O-(*tert*-butyldimethylsilyl)- and 3',5'-bis-O-(*tert*-butyldimethylsilyl) protected nucleosides as colorless foams (2.2 g and 1.0 g, respectively).

Step C: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-*O*-tosyl-β-D-ribofuranosyl)]-4-[di-(4-methoxyphenyl)phenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cooled solution of 2',5'-bis-O-(*tert*-butyldimethylsilyl)-protected nucleoside from Step B (2.0 g, 2.5 mmol) in pyridine (22 mL) was added p-toluenesulfonyl chloride (1.9 g, 9.8 mmol). The reaction mixture was stirred at room temperature for four days. It was then triturated with aqueous pyridine (50%, 10 mL)

and extracted with ether (3 × 50 mL) containing a small amount of CH_2Cl_2 (10 mL). The organic layer was washed with sodium bicarbonate and water (3 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated. Pyridine was removed by co-evaporation with toluene (3 × 25 mL). The residual oil was filtered through a pad of silica gel using hexane:ethyl acetate (70:30) as eluent; yield 1.4 g.

Step D: 4-[di-(4-methoxyphenyl)phenylmethyl]amino-7-[3-*O*-tosyl-β-D-ribofuranosyl-7H-pyrrolo[2,3-*d*]pyrimidine

A solution of the compound from Step C (1.0 g, 1.1 mmol) and THF (10 mL) was stirred with tetrabutylammonium fluoride (1M solution in THF, 2.5 mL) for 0.5h. The mixture was cooled and diluted with ether (50 mL). The solution was washed with water (3 × 50 mL), dried over anhydrous Na_2SO_4, and concentrated to an oil. The residue was purified by passing through a pad of silica gel using hexane: ethyl acetate (1:1) as eluent; yield 780 mg.

Step E: 4-Amino-7-(3-deoxy-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]- pyrimidine and 4-amino-7-(3-deoxy-2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidine

A solution of CH_3MgI (3.0 M solution in ether, 3.0 mL) in anhydrous toluene (3.75 mL) was cooled in an ice bath. To this was added a solution of the compound from Step D (500 mg, 0.8 mmol) in anhydrous toluene (3.7 mL). The resulting mixture was stirred at room temperature for 3.5 h. It was cooled and treated with aqueous NH_4Cl solution and extracted with ether (50 mL containing 10 mL of CH_2Cl_2). The organic layer was separated and washed with brine (2 × 30 mL) and water (2 × 25 mL), dried over anhydrous Na_2SO_4 and concentrated to an oil which was purified by flash chromatography on silica gel using 4% MeOH in CH_2Cl_2 to furnish the 2-C-α-methyl compound (149 mg) and the 2-C-β-methyl compound (34 mg). These derivatives were separately treated with 80% acetic acid and the reaction mixture stirred at room temperature for 2.5 h. The acetic acid was removed by repeated co-evaporation with ethanol and toluene. The residue was partitioned between chloroform and water. The aqueous layer was washed with chloroform and concentrated. The evaporated residue was purified on silica gel using 5-10% MeOH in CH_2Cl_2 as the eluent to furnish the desired compounds as white solids.

4-Amino-7-(3-deoxy-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (9.0 mg):

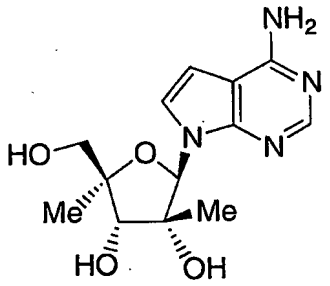
^{1}H NMR (DMSO-d_6): δ 0.74 (s, 3H, CH_3), 1.77 (dd, 1H, H-3'), 2.08 (t, 1H, H-3"), 3.59 (m, 1H, H-5'), 3.73 (m, 1H, H-5"), 4.15 (m, 1H, H-4'), 5.02 (t, 1H, OH-5'), 5.33 (s, 1H, OH-2'), 6.00 (s, 1H, H-1'), 6.54 (d, 1H, H-7), 6.95 (br s, 2H, NH_2), 7.47 (d, 1H, H-8), 8.00 (s, 1H, H-2); ES-MS: 263.1 [M-H].

4-Amino-7-(3-deoxy-2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (15 mg):

^{1}H NMR (DMSO-d_6): δ 1.23 (s, 3H, CH_3), 2.08 (ddd, 2H, H-3'and 3"), 3.57 (m, 2H, H-5'and 5"), 4.06 (m, 1H, H-4), 5.10 (s, 1H, OH-2'), 5.24 (t, 1H, OH-5'), 6.01 (s, 1H, H-1'), 6.49 (d, 1H, H-7),6.89 (br s, 2H, NH_2), 7.35 (d, 1H, H-8), 8.01 (s,1H,H-2). ES-MS: 265.2[M+H].

EXAMPLE 28

4-Amino-7-(2,4-*C*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 5-Deoxy-1,2-*O*-isopropylidene-D-xylofuranose

1,2-*O*-Isopropylidene-D-xylofuranose (38.4 g, 0.2 mol), 4-dimethylaminopyridine (5 g), triethylamine (55.7 mL, 0.4 mol) were dissolved in dichloromethane (300 mL). p-Toluenesulfonyl chloride (38.13 g, 0.2 mol) was added and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was then poured into saturated aqueous sodium bicarbonate (500 mL) and the two layers were separated. The organic layer was washed with aqueous citric acid solution (20%, 200 mL), dried (Na_2SO_4) and evaporated to give a solid (70.0 g). The solid was dissolved in dry THF (300 mL) and $LiAlH_4$ (16.0 g, 0.42 mol) was added in portions over 30 min. The mixture was stirred at room temperature for 15. Ethyl

acetate (100 mL) was added dropwise over 30 min and the mixture was filtered through a silica gel bed. The filtrate was concentrated and the resulting oil was chromatographed on silica gel (EtOAc/hexane 1/4) to afford the product as a solid (32.5 g).

Step B: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-4-methyl-α-D-ribofuranose

Chromium oxide (50 g, 0.5 mol), acetic anhydride (50 mL, 0.53 mol) and pyridine (100 mL, 1.24 mol) were added to dichloromethane (1 L) in an ice-water bath and the mixture was stirred for 15 min. 5-Deoxy-1,2-*O*-isopropylidene-D-xylofuranose (32 g, 0.18 mol) in dichloromethane (200 mL) was added, and the mixture was stirred at the same temperature for 30 min. The reaction solution was diluted with ethyl acetate (1 L) and filtered through a silica gel bed. The filtrate was concentrated to give a yellow oil. The oil was dissolved in 1,4-dioxane (1 L) and formaldehyde (37%, 200 mL). The solution was cooled to 0°C and solid KOH (50 g) was added. The mixture was stirred at room temperature overnight and was then extracted with ethyl acetate (6 × 200 mL). After concentration, the residue was chromatographed on silica gel (EtOAc) to afford the product as an oil (1.5 g). The oil was dissolved in 1-methyl-2-pyrrolidinone (20 mL) and 2,4-dichlorophenylmethyl chloride (4 g, 20.5 mmol) and NaH (60%, 0.8 g) were added. The mixture was stirred overnight and diluted with toluene (100 mL). The mixture was then washed with saturated aqueous sodium bicarbonate (3 × 50 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in methanol (50 mL) and HCl in dioxane (4 M, 2 mL) was added. The solution was stirred overnight and evaporated. The residue was chromatographed on silica gel (EtOAc/hexane:1/4) to afford the desired product as an oil (2.01 g).

Step C: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2,4-di-*C*-methyl-1-*O*-methyl-α-D-ribofuranose

The product (2.0 g, 4.0 mmol) from Step B and Dess-Martin periodinane (2.0 g) in dichloromethane (30 mL) were stirred overnight at room temperature and then concentrated under reduced pressure. The residue was triturated with ether ether (50 mL) and filtered. The filtrate was washed with a solution of $Na_2S_2O_3.5H_2O$ (2.5 g) in saturated aqueous sodium bicarbonate solution (50 mL),

dried ($MgSO_4$), filtered and evaporated. The residue was dissolved in anhydrous Et_2O (20 mL) and was added dropwise to a solution of MeMgBr in Et_2O (3 M, 10 mL) at –78 °C. The reaction mixture was allowed to warm to –30°C and stirred at –30°C to –15°C for 5 h, then poured into saturated aqueous ammonium chloride (50 mL). The two layers were separated and the organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was chromatographed on silica gel (EtOAc/hexane: 1/9) to afford the title compound as a syrup (1.40 g).

Step D: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2,4-di-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step C (0.70 g, 1.3 mmol) was added HBr (5.7 M in acetic acid, 2 mL). The resulting solution was stirred at room temperature for 1 h, evaporated in vacuo and co-evaporated with anhydrous toluene (3 × 10 mL). 4-Chloro-*1H*-pyrrolo[2,3-*d*]pyrimidine (0.5 g, 3.3 mmol) and powdered KOH (85%, 150 mg, 2.3 mmol) were stirred in 1-methyl-2-pyrrolidinone (5 mL) for 30 min and the mixture was co-evaporated with toluene (10 mL). The resulting solution was poured into the above bromo sugar residue and the mixture was stirred overnight. The mixture was diluted with toluene (50 mL), washed with water (3 × 50 mL) and concentrated under reduced pressure. The residue was chromatographed on silica gel eluting with EtOAc/ Hexane (15/85) to afford a solid (270 mg).

Step E: 4-Amino-7-(2,4-*C*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

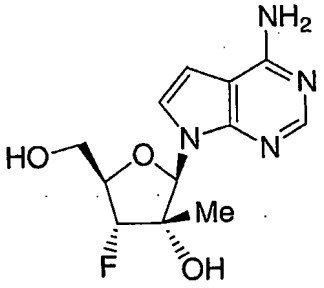
The compound from Step D (270 mg) was dissolved in dioxane (2 mL) and liquid ammonia (20 g) was added in a stainless steel autoclave. The mixture was heated at 100°C for 15, then cooled and evaporated. The residue was chromatographed on silica gel (EtOAc) to afford a solid (200 mg). The solid (150 mg) and Pd/C (10% 150 mg) in methanol (20 mL) were shaken under H_2 (30 psi) for 3 h, filtered and evaporated. The residue was chromatographed on silica gel ($MeOH/CH_2Cl_2$: 1/9) to afford the desired product as a solid (35 mg).
^1H NMR (DMSO-d_6): δ 0.65 (s, 3H), 1.18 (s, 3H), 3.43 (m, 2H), 4.06 (d, 1H, *J* 6.3 Hz), 4.87 (s, 1H), 5.26 (br, 1H), 5.08 (d, 1H, *J* 6.3 Hz), 5.25 (t, 1H, *J* 3.0 Hz), 6.17 (s, 1H), 6.54 (d, 1H, *J* 3.5 Hz), 6.97 (s, br, 2H), 7.54 (d, 1H, *J* 3.4 Hz), 8.02 (s, 1H).

^{13}C NMR (DMSO-d_6): δ 18.19, 21.32, 65.38, 73.00, 79.33, 84.80, 90.66, 99.09, 102.41, 121.90, 149.58, 151.48, 157.38.

LC-MS: Found: 295.1 ($M+H^+$); calculated for $C_{13}H_{18}N_4O_4+H^+$: 295.1.

EXAMPLE 29

4-Amino-7-(3-deoxy-3-fluoro-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 3-Deoxy-3-fluoro-1-*O*-methyl-5-*O*-toluoyl-α-D-ribofuranose

1,2-*O*-Isopropylidene-D-xylofuranose (9.0 g, 50 mmol) and *p*-toluoyl chloride (7.0 mL, 50 mmol) in pyridine (50 mL) were stirred for 30 min. Water (10 mL) was added and the mixture was concentrated under reduced pressure. The residue was dissolved in toluene (500 mL) and the solution was washed with water (200 mL) and saturated aqueous sodium bicarbonate (200 mL). The two layers were separated and the organic layer was evaporated. The residue was dissolved in methanol (100 mL) and HCl in dioxane (4 M, 10 mL) was added. The mixture was stirred at room temperature overnight and was then evaporated under reduced pressure. The resulting oil was chromatographed on silica gel (EtOAc/hexane: 1/1) to afford an oil (10.1 g). The oil was dissolved in dichloromethane (100 mL) and diethylaminosulfur trifluoride (DAST) (5.7 mL) was added. The mixture was stirred overnight and was then poured into saturated aqueous sodium bicarbonate solution (100 mL). The mixture was extracted with toluene (2 × 50 mL) and the combined organic layers were concentrated. The residue was chromatographed on silica gel (EtOAc/hexane: 15/85) to afford the title compound as an oil (1.50 g).

Step B: 3-Deoxy-3-fluoro-2-*C*-methyl-1-*O*-methyl-5-*O*-toluoyl-α-D-ribofuranose

The product from Step A (1.0 g, 3.5 mmol) and Dess-Martin periodinane (2.5 g) in dichloromethane (20 mL) were stirred overnight at room temperature and was then concentrated under reduced pressure. The residue was triturated with diethyl ether (50 mL) and filtered. The filtrate was washed with a solution of $Na_2S_2O_3.5H_2O$ (12.5 g) in saturated aqueous sodium bicarbonate (100 mL), dried ($MgSO_4$), filtered and evaporated. The residue was dissolved in anhydrous THF (50 mL). $TiCl_4$ (3 mL) and methyl magnesium bromide in ethyl ether (3 M, 10 mL) were added at –78°C and the mixture was stirred at –50 to –30°C for 2 h. The mixture was poured into saturated aqueous sodium bicarbonate solution (100 mL) and filtered through Celite. The filtrate was extracted with toluene (100 mL) and evaporated. The residue was chromatographed on silica gel (EtOAc/hexane: 15/85) to afford the title compound as an oil (150 mg).

Step C: 4-Amino-7-(3-deoxy-3-fluoro-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

The product from Step B (150 mg, 0.5 mmol) was dissolved in HBr (30%) in acetic acid (2 mL). After one hour, the mixture was evaporated under reduced pressure and co-evaporated with toluene (10 mL). 4-Chloro-*1H*-pyrrolo[2,3-*d*]pyrimidine (0.5 g, 3.3 mmol) and powdered KOH (85%, 150 mg, 2.3 mmol) were stirred in DMF (3 mL) for 30 min and the mixture was co-evaporated with toluene (2 mL). The resulting solution was poured into the above bromo sugar and the mixture was stirred overnight. The mixture was diluted with toluene (50 mL), washed with water (3 × 50 mL) and concentrated under reduced pressure. The residue was chromatographed on silica gel (EtOAc/hexane: 15/85) to afford an oil (60 mg). The oil was dissolved in dioxane (2 mL) and liquid ammonia (20 g) was added in a stainless steel autoclave. The mixture was heated at 85°C for 18 h, then cooled and evaporated. The residue was chromatographed on silica gel (methanol/dichloromethane: 1/9) to afford the title compound as a solid (29 mg). ^{1}H NMR (DMSO-d_6): δ 0.81 (s, 3H), 3.75 (m, 2H), 4.16 (m, 1H), 5.09 (dd, 1H, *J* 53.2, 7.8 Hz), 5.26 (br, 1H), 5.77 (s, 1H), 6.15 (d, 1H, *J* 2.9 Hz), 6.59 (d, 1H, *J* 3.4 Hz), 7.02 (s br, 2H), 7.39 (d, 1H, *J* 3.4 Hz), 8.06 (s, 1H).

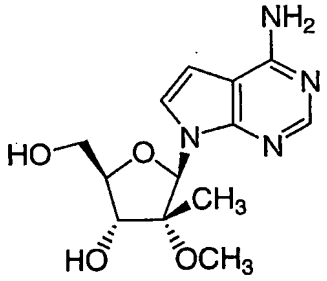
^{13}C NMR (DMSO-d_6): 19.40, 59.56, 77.24, 79.29, 90.15, 91.92, 99.88, 102.39, 121.17, 149.80, 151.77, 157.47.

^{19}F NMR (DMSO-d_6): δ 14.66 (m).

ES-MS: Found: 283.1 ($M+H^+$); calculated for $C_{12}H_{15}FN_4O_3+H^+$: 283.1.

EXAMPLE 30

4-Amino-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a pre-cooled (0°C) solution of the compound from Example 2, Step D (618 mg, 1.0 mmol) in THF (8 mL) was added methyl iodide (709 mg, 5.0 mmol) and NaH (60% in mineral oil) (44 mg, 1.1 mmol). The resulting mixture was stirred overnight at room temperature and then poured into a stirred mixture of saturated aqueous ammonium chloride (50 mL) and dichloromethane (50 mL). The organic layer was washed with water (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The resulting crude product was purified on silica gel using ethyl acetate/hexane as the eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (735 mg) as a colorless foam.

Step B: 4-amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step A (735 mg, 1.16 mmol) was added methanolic ammonia (saturated at 0°C) (20 mL). The mixture was heated in a stainless steel autoclave at 80°C overnight, then cooled and the content evaporated in vacuo. The crude mixture was purified on silica gel using ethyl acetate/hexane as the

eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (504 mg) as colorless foam.

Step C: 4-amino-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

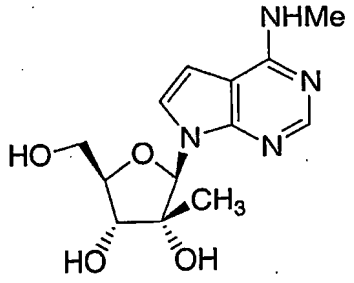
A mixture of the product from Step C (64 mg, 0.1 mmol), MeOH (5 mL), Et_3N (0.2 mL) and 10% Pd/C (61 mg) was hydrogenated on a Parr hydrogenator at 50 psi at room temperature overnight. The mixture was filtered throught celite, evaporated in vacuo and filtered through a pad of silica gel using 2% methanol in dichloromethane as eluent. The desired product was collected and evaporated in vacuo. The compound was redissolved in methanol (10 mL) and 10% Pd/C (61 mg) was added. The mixture was hydrogenated on a Parr hydrogenator at 55 psi at room temperature for two weeks. The mixture was filtered through celite, evaporated in vacuo and purified on silica gel using 10% methanol in dichloromethane as eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (110 mg) as a colorless foam.

^1H NMR (DMSO-d_6): δ 0.68 (s, 3H,), 3.40 (s, 3H), 3.52-3.99 (overlapping m, 4H), 4.92 (d, 1H), 5.07 (t, 1H), 6.26 (s, 1H), 6.55 (d, 1H), 7.00s br, 2H), 7.46 (d, 1H), 8.05 (s, 1H).

LC-MS: Found: 293.1 ($M-H^+$); calc. for $C_{12}H_{16}N_4O_4-H^+$: 293.12.

EXAMPLE 31

4-Methylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

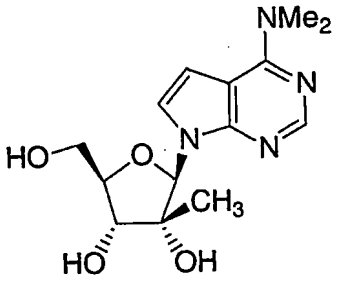


The compound from Step E of Example 2 (200 mg, 0.67 mmol) was added to methylamine (5 mL condensed in a small stainless steel autoclave) and warmed at 85°C for 48 h, then cooled and evaporated in vacuo. The crude mixture

was purified on a silica gel with ethanol as the eluent to give the title compound which separated as an amorphous solid after treatment with MeCN. The amorphous solid was dissolved in water and lyophilized to give a colorless powder (144 mg).
^{1}H NMR (DMSO-d_6): δ 0.63 (s, 3H, CH_3), 3.32 (s, 3H, N CH_3), 3.58-3.67 (m, 1H, H-5'), 3.79-3.39 (m, 3H, H-5", H-4', H-3'), 5.03 (s, 1H, 2'-OH), 5.04-5.11 (1H,3'-OH, 1H, 5'-OH), 6.14 (s, 1H, H-1'), 6.58 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 7.46 (d, 1H, H-6), 7.70 (br s, 1H, NH), 8.14 (s, 1H, H-2).
LC-MS: Found: 295.1 (M-H$^+$); calc. for $C_{13}H_{18}N_4O_4$+H$^+$: 294.3.

EXAMPLE 32

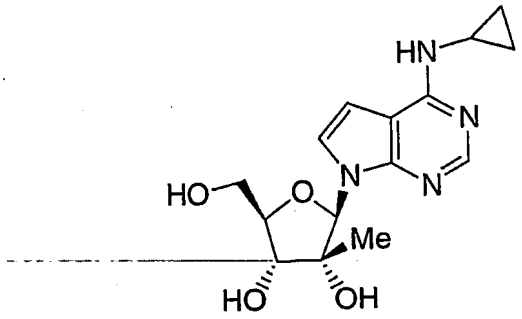
4-Dimethylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



The compound from Step E of Example 2 (200 mg, 0.67 mmol) was added to dimethylamine (5 mL condensed in a small stainless steel autoclave) and warmed at 85°C for 48 h, then cooled and evaporated in vacuo. The crude mixture was purified on a silica gel with ethanol as the eluent to give the title compound which separated as an amorphous solid after treatment with MeCN. The amorphous solid was dissolved in water and lyophilized to give a colorless powder (164 mg).
^{1}H NMR (DMSO-d_6): δ 0.64 (s, 3H, CH_3), 3.29 (s, 3H, N CH_3), 3.32 (s, 3H, N CH_3), 3.60-3.66 (m, 1H, H-5'), 3.77-3.97 (m, 3H, H-5", H-4', H-3'), 5.04 (s, 1H, 2'-OH), 5.06-5.11 (1H, 3'-OH, 1H, 5'-OH), 6.21 (s, 1H, H-1'), 6.69 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 7.55 (d, 1H, H-6), 8.13 (s, 1H, H-2).
LC-MS: Found: 309.3 (M-H$^+$); calc. for $C_{14}H_{20}N_4O_4$+H$^+$: 308.33.

EXAMPLE 33

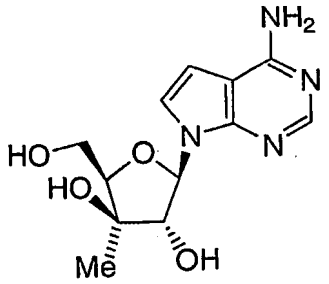
4-Cyclopropylamino-7-(2-C-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



The compound from Step E of Example 2 (200 mg, 0.67 mmol) was added to cyclopropylamine (5 mL condensed in a small stainless steel autoclave) and warmed at 85°C for 48 h, then cooled and evaporated in vacuo. The crude mixture was purified on a silica gel with ethanol as the eluent to give the title compound which separated as an amorphous solid after treatment with MeCN. The amorphous solid was dissolved in water and lyophilized to give a colorless powder (148 mg).
^{1}H NMR (DMSO-d_6): δ 0.51- 0.58 (m, 2H), 0.64 (s, 3H, CH_3), 0.74- 0.076 (m, 2H), 3.62-3.67 (m, 1H, H-5'), 3.79-3.82 (m, 3H, H-5"), 3.92-3.96 (m, H-4', H-3'), 5.03 (s, 1H, 2'-OH), 5.05-5.10 (1H, 3'-OH, 1H, 5'-OH), 6.15 (s, 1H, H-1'), 7.48 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 7.59 (d, 1H, H-6), 8.13 (s, 1H, H-2).
LC-MS: Found: 321.1 ($M-H^+$); calc. for $C_{15}H_{20}N_4O_4+H^+$: 320.3.

EXAMPLE 34

4-Amino-7-(3-*C*-methyl-β-D-xylofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)]-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine and 7-[3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of mixture of the compounds from Step A of Examples 26 and 27 (0.32 g, 0.65 mmol) in anhydrous pyridine (6 mL) was added monomethoxytrityl chloride (0.30 g, 0.98 mmol) and the reaction mixture was stirred at room temperature overnight. The mixture was then concentrated and the residue was partitioned between CH_2Cl_2 (70 mL) and water (20 mL). The organic layer was washed with water and brine, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel column using 5-13% EtOAc in hexanes as the eluent. The appropriate fractions were collected and concentrated to furnish 2',5'-bis-O-(*tert*-butyldimethylsilyl)- and 3',5'-bis-O-(*tert*-butyldimethylsilyl) protected nucleosides as colorless foams (343 mg and 84 mg, respectively).

Step B: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pentofuranos-3-ulosyl]-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a well-stirred suspension of chromium trioxide (91 mg, 0.91 mmol) in CH_2Cl_2 (4 mL) at 0°C were added pyridine (147 μL, 1.82 mmol) and then acetic anhydride (86 μL, 0.91 mmol). The mixture was stirred at room temperature for 0.5 h. Then the 2',5'-bis-O-(*tert*-butyldimethylsilyl) protected nucleoside from step A (343 mg 0.45 mmol) in CH_2Cl_2 (2.5 mL) was added and the mixture stirred at room temperature 2 h. The mixture was then poured into ice-cold EtOAc (10 mL) and filtered through a short silica gel column using EtOAc as the eluent. The filtrate was evaporated and the residue purified on a silica gel column with hexanes and hexanes/EtOAc (7/1) as the eluent to give the title compound (180 mg).

Step C: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-*C*-methyl-β-D-ribofuranosyl)-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine and 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-*C*-methyl-β-D-xylofuranosyl)-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a mixture of MeMgBr (3.0 M solution in ether; 0.17 mL, 0.5 mmol) in anhydrous hexanes (1.5 mL) at room temperature was added dropwise a solution of the compound from Step B (78 mg, 0.1 mmol) in anhydrous hexanes (0.5 mL). After 2 h stirring at room temperature, the reaction mixture was poured into ice-cold water (10 mL) and diluted with EtOAc (20 mL), then filtered through Celite which was then thoroughly washed with EtOAc. The layers were separated and the organic layer was

washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified on a silica gel column using 8 to 25% EtOAc in hexanes as eluent to give the 3-C-methyl xylo- (60 mg) and the 3-C-methyl ribo-isomer (20 mg).

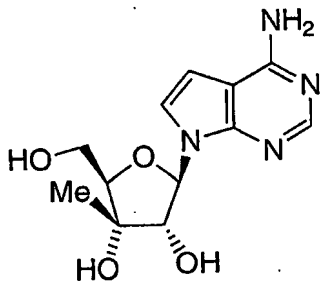
Step D: 4-Amino-7-(3-*C*-methyl-β-D-xylofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold solution of 3-C-methyl-xylo isomer from Step C (60 mg, 0.08 mmol) in THF (2 mL) was added TBAF (1 M in THF; 0.32 mL, 0.32 mmol). The reaction mixture was stirred at room temperature for 5 h, then diluted with CH_2Cl_2 (50 mL), washed with water (3 × 15 mL), dried,and evaporated. The residue was dissolved in dioxane (0.3 mL) and 80% acetic acid (3 mL) was added. The reaction mixture was stirred at room temperature for 24 h and then evaporated. The residue was co-evaporated with dioxane, taken up in water (50 mL) and washed with CH_2Cl_2 (2 × 10 mL). The aqueous layer was concentrated and then freeze-dried. The residue was purified on silica gel column with CH_2Cl_2/MeOH (20/1 and 10/1) as the eluent to give the title compound as a white fluffy compound after freeze drying (10 mg).

^1H NMR (CD_3CN): δ 1.28 (s, 3H, CH_3), 3.56 (br s, 1H, OH), 3.78 (m, 3H, H-4', H-5', H-5"), 4.10 (br s, 1H, OH), 4.44 (d, 1H, $J_{2',1'}$ = 3.9 Hz, H-2'), 5.58 (d, 1H, H-1'), 5.85 (br s, 2H, NH_2), 6.15 (br s, 1H, OH), 6.48 (d, 1H, $J_{5,6}$ = 3.7 Hz, H-5), 7.23 (d, 1H, H-6), 8.11 (s, 1H, H-2). ES-MS: 281 $[MH]^+$.

EXAMPLE 35

4-Amino-7-(3-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

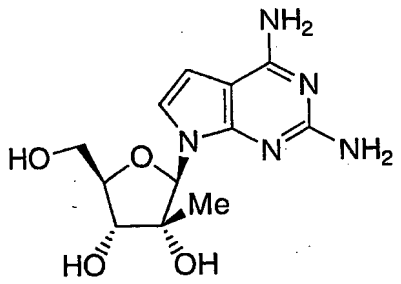


The ribo-isomer (20 mg) from Step C of Example 32 was deprotected using the procedure described in Step D of Example 32 to yield the title compound (4 mg).

^{1}H NMR (CD_3CN): δ 1.43 (s, 3H, CH_3), 3.28 (br s, 1H, OH), 3.58 (m, 2H, H-5', H-5"), 3.99 (m, 1H, H-4'), 4.10 (br s, 1H, OH), 4.62 (d, 1H, $J_{2'1'}$ = 8.1 Hz, H-2'), 5.69 (d, 1H, H-1'), 5.88 (br s, 3H, OH, NH_2), 6.45 (br s, 1H, OH), 6.51 (d, 1H, $J_{5,6}$ = 3.7 Hz, H-5), 7.19 (d, 1H, H-6), 8.12 (s, 1H, H-2). ES-MS: 281 $[MH]^+$.

EXAMPLE 36

2,4-Diamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



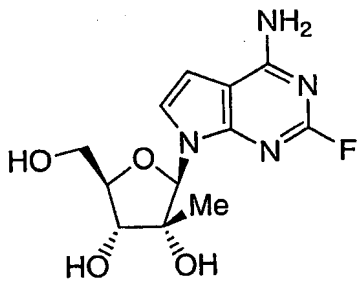
A mixture of the product from Step B of Example 4 (24 mg) in aqueous ammonia (30%, 10 mL) was heated in a stainless steel autoclave at 100 °C overnight, then cooled and evaporated. The residue was purified on a silica gel column with CH_2Cl_2/MeOH (10/1 and 5/1) as the eluent to afford the title compound (15 mg).

^{1}H NMR (DMSO-d_6): δ 0.68 (s, 3H, CH_3), 3.48-3.58 (m 1H, H-5'), 3.68-3.73 (m, 2H, H-5", H-4'), 3.84 (m, 1H, H-3'), 4.72 (s, 1H, 2'-OH), 4.97-5.03 (m, 2H, 3'-OH, 5'-OH), 5.45 (br s, 2H, NH_2), 6.00 (s, 1H, H-1'), 6.28 (d, 1H, J = 3.7 Hz, H-5), 6.44 (br s, 2H, NH_2) 6.92 (d, 1H J = 3.7 Hz, H-6).

ES MS: 294.1 ($M-H^+$).

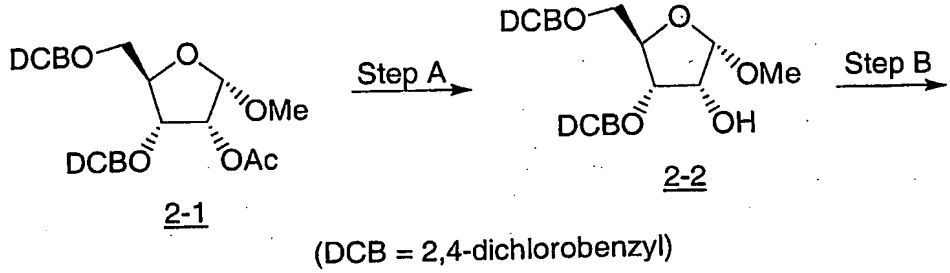
EXAMPLE 37

4-Amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



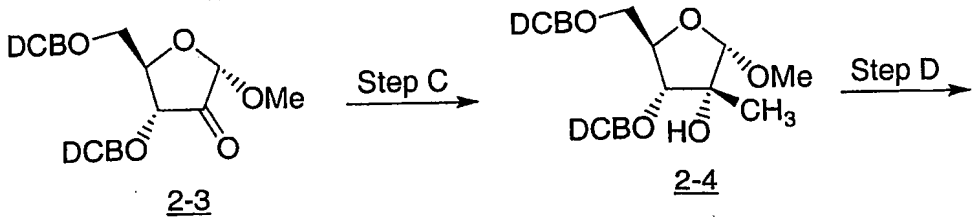
To a solution of HF/pyridine (70%, 2 mL) diluted with pyridine (1 mL) at –30 °C is added the compound of Example 36 (60 mg, 0.2 mmol) in 0.5 mL pyridine followed by *tert*-butyl nitrite (36 μL, 0.3 mmol). Stirring is continued for 5 min at –25 °C. Then the solution is poured into ice-water (5 mL), neutralized with 2 N aqueous NaOH, and evaporated to dryness. The residue is purified on a silica gel column with CH_2Cl_2/MeOH (20/1 and 10/1) as the eluent to afford the title compound.

Scheme 2

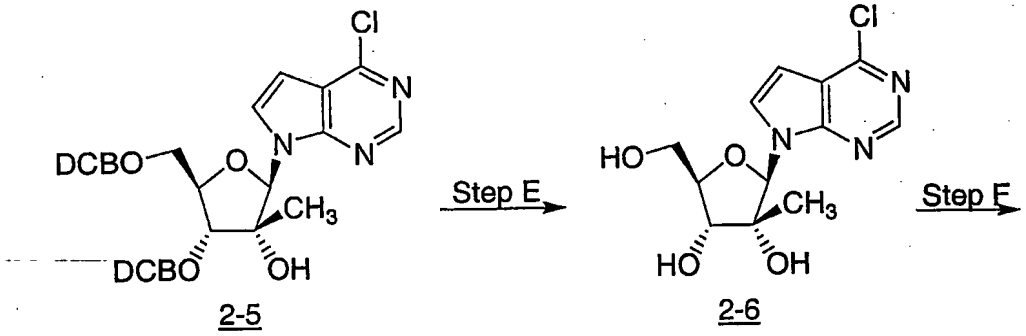


2-1 2-2

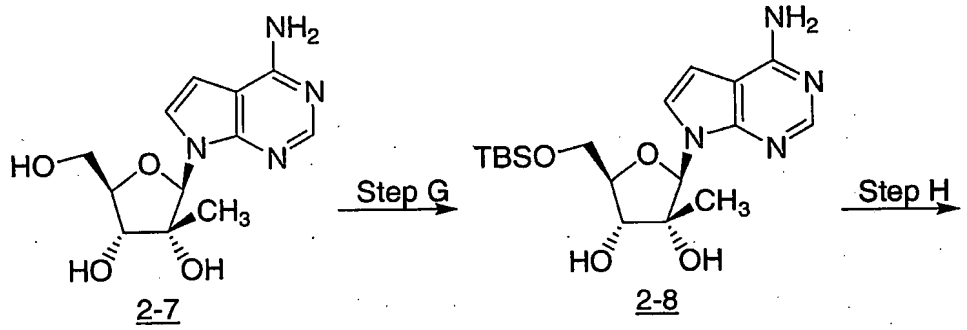
(DCB = 2,4-dichlorobenzyl)



2-3 2-4

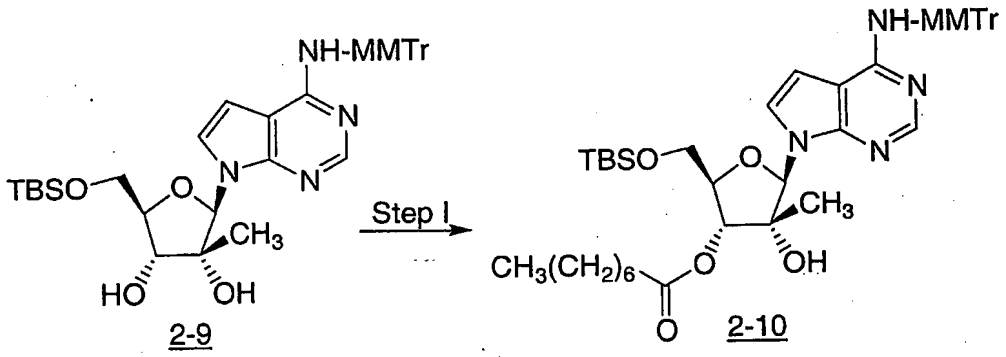


2-5 2-6



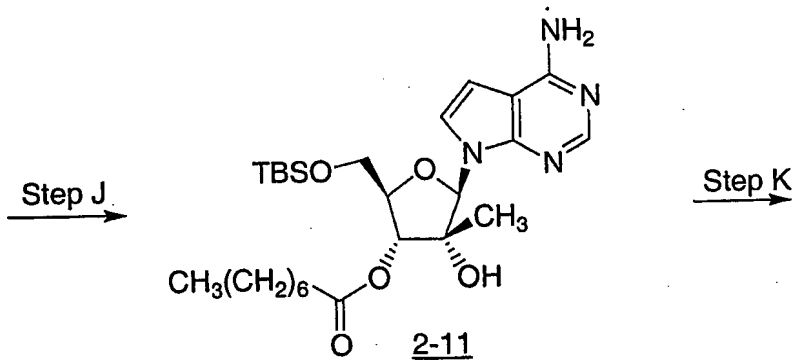
2-7 2-8

(TBS = *tert*-butyldimethylsilyl)

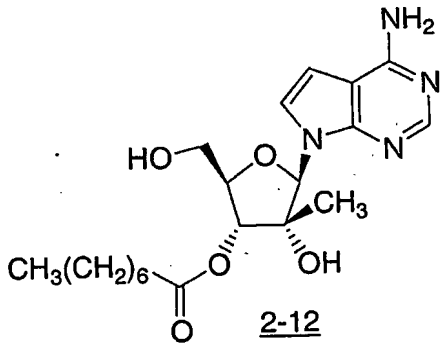


2-9 2-10

(MMTr = p-methoxyphenyldiphenylmethyl)



2-11



2-12

EXAMPLE 38

4-Amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-12)

Step A: 3,5-Bis-*O*-(2,4-dichlorobenzyl)-1-*O*-methyl-α-D-ribofuranose (1-2)

A mixture of 2-*O*-acetyl-3,5-bis-*O*-(2,4-dichlorobenzyl)-1-*O*-methyl-α-D-ribofuranose (2-1) [for preparation, see: Helv. Chim. Acta 78: 486 (1995)] (52.4 g, 0.10 mol) in methanolic K_2CO_3 (500 mL, saturated at room temperature) was stirred at room temperature for 45 min. and then concentrated under reduced pressure. The oily residue was suspended in CH_2Cl_2 (500 mL), washed with water (300 mL + 5 × 200 mL) and brine (200 mL), dried (Na_2SO_4), filtered, and concentrated to give the title compound (49.0 g) as colorless oil, which was used without further purification in Step B below.

^{1}H NMR (DMSO-d_6): δ 3.28 (s, 3H, OCH_3), 3.53 (d, 2H, $J_{5,4}$ = 4.5 Hz, H-5a, H-5b), 3.72 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,2}$ = 6.6 Hz, H-3), 3.99 (ddd, 1H, $J_{2,1}$ = 4.5 Hz, $J_{2,OH-2}$ = 9.6 Hz, H-2), 4.07 (m, 1H, H-4), 4.50 (s, 2H, CH_2Ph), 4.52, 4.60 (2d, 2H, J_{gem} = 13.6

Hz, CH_2Ph), 4.54 (d, 1H, OH-2), 4.75 (d, 1H, H-1), 7.32-7.45, 7.52-7.57 (2m, 10H, 2Ph).

^{13}C NMR (DMSO-d_6) δ 55.40, 69.05, 69.74, 71.29, 72.02, 78.41, 81.45, 103.44, 127.83, 127.95, 129.05, 129.28, 131.27, 131.30, 133.22, 133.26, 133.55, 133.67, 135.45, 135.92.

Step B: 3,5-Bis-*O*-(2,4-dichlorobenzyl)-1-*O*-methyl-α-D-*erythro*-pentofuranos-2-ulose (2-3)

To an ice-cold suspension of Dess-Martin periodinane (50.0 g, 118 mmol) in anhydrous CH_2Cl_2 (350 mL) under argon (Ar) was added a solution of the compound from Step A (36.2 g, 75 mmol) in anhydrous CH_2Cl_2 (200 mL) dropwise over 0.5 h. The reaction mixture was stirred at 0°C for 0.5 h and then at room temperature for 3 days. The mixture was diluted with anhydrous Et_2O (600 mL) and poured into an ice-cold mixture of $Na_2S_2O_3.5H_2O$ (180 g) in saturated aqueous $NaHCO_3$ (1400 mL). The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (600 mL), water (800 mL) and brine (600 mL), dried ($MgSO_4$), filtered and evaporated to give the title compound (34.2 g) as a colorless oil, which was used without further purification in Step C below.

^{1}H NMR ($CDCl_3$): δ 3.50 (s, 3H, OCH_3), 3.79 (dd, 1H, $J_{5a,5b}$ = 11.3 Hz, $J_{5a,4}$ = 3.5 Hz, H-5a), 3.94 (dd, 1H, $J_{5b,4}$ = 2.3 Hz, H-5b), 4.20 (dd, 1H, $J_{3,1}$ = 1.3 Hz, $J_{3,4}$ = 8.4 Hz, H-3), 4.37 (ddd, 1H, H-4), 4.58, 4.69 (2d, 2H, J_{gem} = 13.0 Hz, CH_2Ph), 4.87 (d, 1H, H-1), 4.78, 5.03 (2d, 2H, J_{gem} = 12.5 Hz, CH_2Ph), 7.19-7.26, 7.31-7.42 (2m, 10H, 2Ph).

^{13}C NMR (DMSO-d_6): δ 55.72, 69.41, 69.81, 69.98, 77.49, 78.00, 98.54, 127.99, 128.06, 129.33, 129.38, 131.36, 131.72, 133.61, 133.63, 133.85, 133.97, 134.72, 135.32, 208.21.

Step C: 3,5-Bis-*O*-(2,4-dichlorobenzyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose (2-4)

To a solution of MeMgBr in anhydrous Et_2O (0.48 M, 300 mL) at –55 °C was added dropwise a solution of the compound from Step B (17.40 g, 36.2 mmol) in anhydrous Et_2O (125 mL). The reaction mixture was allowed to warm to –30°C and stirred for 7 h at –30°C to –15°C, then poured into ice-cold water (500 mL) and the mixture vigorously stirred at room temperature for 0.5 h. The mixture was filtered through a Celite pad (10 × 5 cm) which was thoroughly washed with Et_2O.

The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in hexanes (~30 mL), applied onto a silica gel column (10 × 7 cm, prepacked in hexanes) and eluted with hexanes and hexanes/EtOAc (9/1) to give the title compound (16.7 g) as a colorless syrup.

^{1}H NMR ($CDCl_3$): δ 1.36 (d, 3H, $J_{Me,OH}$ = 0.9 Hz, 2C-Me), 3.33 (q, 1H, OH), 3.41 (d, 1H, $J_{3,4}$ = 3.3 Hz), 3.46 (s, 3H, OCH_3), 3.66 (d, 2H, $J_{5,4}$ = 3.7 Hz, H-5a, H-5b), 4.18 (apparent q, 1H, H-4), 4.52 (s, 1H, H-1), 4.60 (s, 2H, C*H*$_2$Ph), 4.63, 4.81 (2d, 2H, J_{gem} = 13.2 Hz, C*H*$_2$Ph), 7.19-7.26, 7.34-7.43 (2m, 10H, 2Ph).

^{13}C NMR ($CDCl_3$): δ 24.88, 55.45, 69.95, 70.24, 70.88, 77.06, 82.18, 83.01, 107.63, 127.32, 129.36, 130.01, 130.32, 133.68, 133.78, 134.13, 134.18, 134.45, 134.58.

Step D: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorobenzyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-5)

To a solution of the compound from Step C (9.42 g, 19 mmol) in anhydrous dichloromethane (285 mL) at 0°C was added HBr (5.7 M in acetic acid, 20 mL, 114 mmol) dropwise. The resulting solution was stirred at 0°C for 1 h and then at room temperature for 3h, evaporated *in vacuo* and co-evaporated with anhydrous toluene (3 × 40 mL). The oily residue was dissolved in anhydrous acetonitrile (50 mL) and added to a solution of sodium salt of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see J. Chem. Soc., 131 (1960)] in acetonitrile [generated *in situ* from 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine (8.76 g, 57 mmol) in anhydrous acetonitrile (1000 mL), and NaH (60% in mineral oil, 2.28 g, 57 mmol), after 4 h of vigorous stirring at room temperature]. The combined mixture was stirred at room temperature for 24 h, and then evaporated to dryness. The residue was suspended in water (250 mL) and extracted with EtOAc (2 × 500 mL). The combined extracts were washed with brine (300 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified on a silica gel column (10 cm × 10 cm) using ethyl acetate/hexane (1:3 and 1:2) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product (5.05 g) as a colorless foam.

^{1}H NMR ($CDCl_3$): δ 0.93 (s, 3H, CH_3), 3.09 (s, 1H, OH), 3.78 (dd, 1H, $J_{5',5''}$ = 10.9 Hz, $J_{5',4}$ = 2.5 Hz, H-5'), 3.99 (dd, 1H, $J_{5'',4}$ = 2.2 Hz, H-5''), 4.23-4.34 (m, 2H, H-3', H-4'), 4.63, 4.70 (2d, 2H, J_{gem} = 12.7 Hz, C*H*$_2$Ph), 4.71, 4.80 (2d, 2H, J_{gem} = 12.1 Hz,C*H*$_2$Ph), 6.54 (d, 1H, , $J_{5,6}$ = 3.8 Hz, H-5), 7.23-7.44 (m, 10H, 2Ph).

^{13}C NMR ($CDCl_3$): δ 21.31, 69.10, 70.41, 70.77, 79.56, 80.41, 81.05, 91.11, 100.57, 118.21, 127.04, 127.46, 127.57, 129.73, 129.77, 130.57, 130.99, 133.51, 133.99, 134.33, 134.38, 134.74, 135.21, 151.07, 151.15 152.47.

Step E: 4-Chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-6)

To a solution of the compound from Step D (5.42 g, 8.8 mmol) in dichloromethane (175 mL) at -78°C was added boron trichloride (1M in dichloromethane, 88 mL, 88 mmol) dropwise. The mixture was stirred at –78°C for 2.5 h, then at –30°C to –20°C for 3 h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (90 mL) and the resulting mixture stirred at –15°C for 30 min., then neutralized with aqueous ammonia at 0°C and stirred at room temperature for 15 min. The solid was filtered and washed with CH_2Cl_2/MeOH (1/1, 250 mL). The combined filtrate was evaporated, and the residue was purified by flash chromatography over silica gel using CH_2Cl_2 and CH_2Cl_2:MeOH (99:1, 98:2, 95:5 and 90:10) gradient as the eluent to furnish desired compound (1.73 g) as a colorless foam, which turned into an amorphous solid after treatment with MeCN.

^{1}H NMR (DMSO-d_6): δ 0.64 (s, 3H, CH_3), 3.61-3.71 (m, 1H, H-5'), 3.79-3.88 (m, 1H, H-5"), 3.89-4.01 (m, 2H, H-3', H-4'), 5.15-5.23 (m, 3H, 2'-OH, 3'-OH, 5'-OH), 6.24 (s, 1H, H-1'), 6.72 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-5), 8.13 (d, 1H, H-6), 8.65 (s, 1H, H-2).

^{13}C NMR (DMSO-d_6): δ 20.20, 59.95, 72.29, 79.37, 83.16, 91.53, 100.17, 117.63, 128.86, 151.13, 151.19, 151.45.

Step F: 4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-7)

To the compound from Step E (1.54 g, 5.1 mmol) was added methanolic ammonia (saturated at 0°C; 150 mL). The mixture was heated in a stainless steel autoclave at 85°C for 14 h, then cooled and evaporated *in vacuo*. The crude mixture was purified on a silica gel column with CH_2Cl_2/MeOH (9/1) as eluent to give the title compound as a colorless foam (0.8 g), which separated as an amorphous solid after treatment with MeCN. The amorphous solid was recrystallized from methanol/acetonitrile; m.p. 222°C.

^{1}H NMR (DMSO-d_6): δ 0.62 (s, 3H, CH_3), 3.57-3.67 (m, 1H, H-5'), 3.75-3.97 (m, 3H, H-5", H-4', H-3'), 5.00 (s, 1H, 2'-OH), 5.04 (d, 1H, $J_{3'OH,3'}$ = 6.8 Hz, 3'-OH), 5.06 (t, 1H, $J_{5'OH,5',5"}$ = 5.1 Hz, 5'-OH), 6.11 (s, 1H, H-1'), 6.54 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 6.97 (br s, 2H, NH_2), 7.44 (d, 1H, H-6), 8.02 (s, 1H, H-2).

^{13}C NMR (DMSO-d_6): δ 20.26, 60.42, 72.72, 79.30, 82.75, 91.20, 100.13, 103.08, 121.96, 150.37, 152.33, 158.15.

LC-MS: Found: 279.10 ($M-H^+$); calc. for $C_{12}H_{16}N_4O_4+H^+$: 279.11.

Step G: 4-Amino-7-[5-*O*-(*tert*-butyldimethylsilyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-8)

To a solution of the compound from Step F (457 mg, 1.63 mmol) in anhydrous pyridine (3.5 mL) was added *tert*-butyldimethylsilyl chloride (370 mg, 2.45 mmol). The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was then diluted with ethyl acetate (40 mL) which was washed with saturated aqueous sodium bicarbonate solution (20 mL). The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and evaporated to an oil that was subjected to chromatography on silica gel eluting with 10% MeOH in CH_2Cl_2. The appropriate fractions were collected, evaporated, and dried under high vacuum to furnish the title compound as a colorless foam (516 mg).

^{1}H NMR (DMSO-d_6): δ 7.95 (s, 1H), 7.35 (d, 1H, J = 3.4Hz), 6.89 (bs, 2H, NH_2), 6.44 (d, 1H, J = 3.4Hz), 6.02 (s, 1H), 5.01-4.98 (m, 2H), 3.92-3.70 (m, 3H), 3.40-3.25 (m, 1H), 0.82 (s, 9H), 0.54 (s, 3H), 0.00 (s, 6H).

Step H: 4-(p-Methoxyphenyldiphenylmethylamino)-7-[5-*O*-(*tert*-butyldimethylsilyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-9)

To a solution of the compound from Step G (394 mg, 1.0 mmol) in anhydrous pyridine (5 mL) was added p-methoxyphenylchlorodiphenylmethane (946 mg, 3.06 mmol) and 4-dimethylaminopyridine (DMAP) (123 mg, 1.0 mmol). The reaction mixture was stirred at room temperature for 20 h. It was then diluted with ethyl acetate (30 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 15 mL) followed by water (2 x 15mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to an oil. The crude product was purified using column chromatography on silica gel eluting with 5% MeOH in CH_2Cl_2. The

appropriate fractions were collected and evaporated to give the title compound (540 mg).

^{1}H NMR (DMSO-d_6): δ 7.85 (s, 1H), 7.65 (s, 1H), 7.41 (d, 1H, J = 3.8Hz), 7.25-7.03 (m, 12H), 6.78 (d, 1H, J = 3.6 Hz), 6.69 (d, 2H, J = 9 Hz), 5.97 (s, 1H), 5.00-4.94 (m, 2H), 3.85-3.62 (m, 4H), 3.59 (s, 3H), 0.83 (s, 9H), 0.55 (s, 3H), 0.003 (s, 6H).

Step I: 4-(p-Methoxyphenyldiphenylmethylamino)-7-[5-*O*-(*tert*-butyldimethylsilyl)-3-*O*-(1-oxo-octyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-10)

To a solution of the compound from Step H (400 mg, 0.6 mmol) and anhydrous DMAP (73 mg, 0.6 mmol) in anhydrous CH_2Cl_2 (7 mL) was added slowly triethylamine (250 μL, 1.8 mmol). To the stirred solution was added octanoyl chloride (200 μL, 1.2 mmol) over 15 min. The reaction mixture was stirred for an additional 1.5 h. It was then diluted with methylene chloride (30 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 10 mL) and water (10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated. The residue was subjected to column chromatography on silica gel eluting with 5% MeOH in CH_2Cl_2 to afford the title compound as a light yellow foam (340 mg).

^{1}H NMR (DMSO-d_6): δ 8.02 (s, 1H), 7.75 (s, 1H), 7.58 (d, 1H, J = 3.6 Hz), 7.34-7.05 (m, 12H), 7.02 (d, 1H, J = 3.6 Hz), 6.79 (d, 2H, J = 9.0 Hz), 6.01 (s, 1H), 5.61 (s, 1H), 5.34 (d, 1H, J = 9.0 Hz), 4.19-4.14 (m, 1H), 4.00-3.94 (m, 1H), 3.67-3.62 (m, 4H), 3.48-3.40 (m, 1H), 2.40-2.32 (m, 2H), 1.60-1.40 (m, 2H), 1.23 (bs, 8H), 0.91 (s, 9H), 0.84-0.80 (m, 3H), 0.67 (s, 3H), 0.07 (s, 6H).

Step J: 4-Amino-7-[5-*O*-(*tert*-butyldimethylsilyl)-3-*O*-(1-oxo-octyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-11)

A solution of the compound from Step I (250 mg, 0.31 mmol) in 6:3:1 MeOH:acetic acid:H_2O (10 mL) was stirred at 50°C for 12 h. The reaction mixture was then concentrated to dryness. The residue was diluted with ethyl acetate (30 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 15 mL) and water (2 x 10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated. The crude product (200 mg) was used without further purification in Step K below. Further purification of a small amount was accomplished by silica gel column chromatography using 5% MeOH in CH_2Cl_2 as the eluent to give the title compound as a white foam .

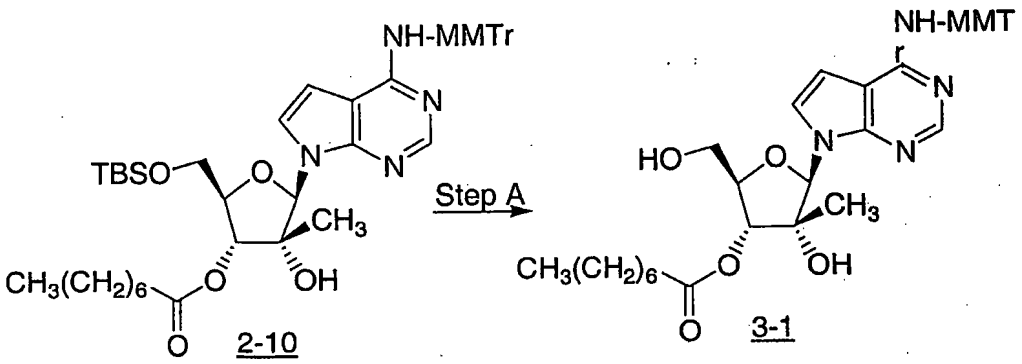
^{1}H NMR ($CDCl_3$): δ 8.29 (s,1H), 7.57 (d, 1H, J = 3.8 Hz), 6.37 (d, 1H, J = 3.8 HZ), 6.28 (s, 1H), 5.33-5.28 (m, 3H), 4.29-4.23 (m, 1H), 4.08-4.01 (m, 1H), 3.86-3.79 (m,1H), 2.45-2.37 (m, 2H), 1.69-1.62 (m, 2H), 1.29-1.23 (m,8H), 0.97-0.84 (m, 12H), 0.11 (s, 6H).

Step K: 4-Amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-12)

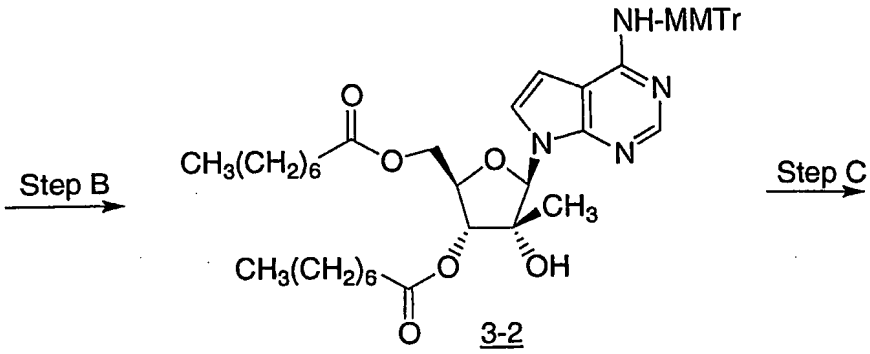
To a solution of the compound from Step J (230 mg, 0.44 mmol) in anhydrous THF (5mL), was added triethylamine (300 µL, 2.14 mmol) and triethylamine trihydrofluoride (750 µL, 4.5 mmol). The solution was stirred overnight at room temperature. The reaction mixture was then diluted with ethyl acetate (30 mL) and washed with saturated aqueous sodium bicarbonate (3 x 10 mL) and water (10 mL). After drying the organic layer over anhydrous sodium sulfate and filtration, the solvent was evaporated. The resulting oil was purified on a silica gel column eluting with 1:1 acetone/CH_2Cl_2 followed by 10% MeOH in CH_2Cl_2. The appropriate fractions were concentrated and lyophilized to afford the title compound as a colorless powder (90 mg).

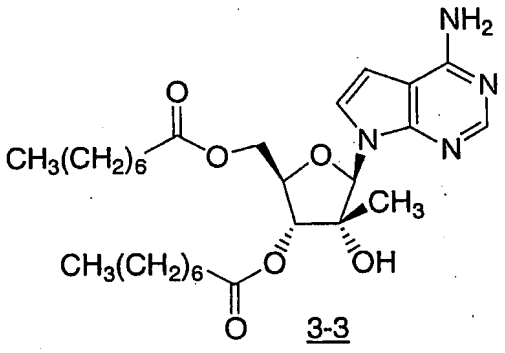
^{1}H NMR ($CDCl_3$): δ 8.30 (s, 1H), 7.31 (d, 1H, J = 3.8 Hz), 6.39 (d, 1H, J = 3.8 Hz), 6.16 (s, 1H), 5.44 (d, 1H, J = 7.8 Hz), 5.23 (bs, 2H), 4.31-4.24 (m, 1H), 4.14-4.06 (m, 1H), 3.84-3.76 (m, 1H), 2.48-2.40 (m, 2H), 1.80-1.50 (m, 3H), 1.34-1.23 (m, 7H), 0.95 (s, 3H), 0.88-0.55 (m, 3H).

Scheme 3



(MMTr = p-methoxyphenyldiphenylmethyl)





EXAMPLE 39

4-Amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3-3)

Step A: 4-(p-Methoxyphenyldiphenylmethylamino)-7-[3-*O*-(1-oxo-octyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3-1)

A solution of the compound from Step I of Example 1 (1-10) (300 mg, 0.37 mmol), anhydrous triethylamine (300 μL, 2.14 mmol) and triethylamine trihydrofluoride (750 μL, 4.5 mmol) in anhydrous THF (5 mL) was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 20 mL) followed by water (2 x 15 mL). The organic layer was separated, dried over sodium sulfate, filtered, and evaporated. The crude product was purified on a silica gel column using 10-15% acetone in CH_2Cl_2 as the eluent. The appropriate fractions were combined and evaporated to afford the title compound as a colorless foam (240 mg).

^{1}H NMR (DMSO-d_6): δ 8.03 (s, 1H), 7.79 (s, 1H), 7.56 (d, 1H, J = 3.8 Hz), 7.38-7.17 (m, 12H), 7.04 (d, 1H, J = 3.8 Hz), 6.83 (d, 2H, J = 9.0 Hz), 6.13 (s, 1H), 5.56 (s, 1H), 5.31 (d, 1H, J = 9 Hz), 5.21-5.16 (m, 1H), 4.20-4.08 (m, 1H), 3.38-3.70 (m, 4H), 3.65-3.40 (m, 2H), 2.43-2.36 (m, 2H), 1.63-1.45 (m, 2H), 1.27 (bs, 8H), 0.91-0.84 (m, 3H), 0.74 (s, 3H).

Step B: 4-(p-Methoxyphenyldiphenylmethylamino)-7-[3,5-di-*O*-(1-oxo-octyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3-2)

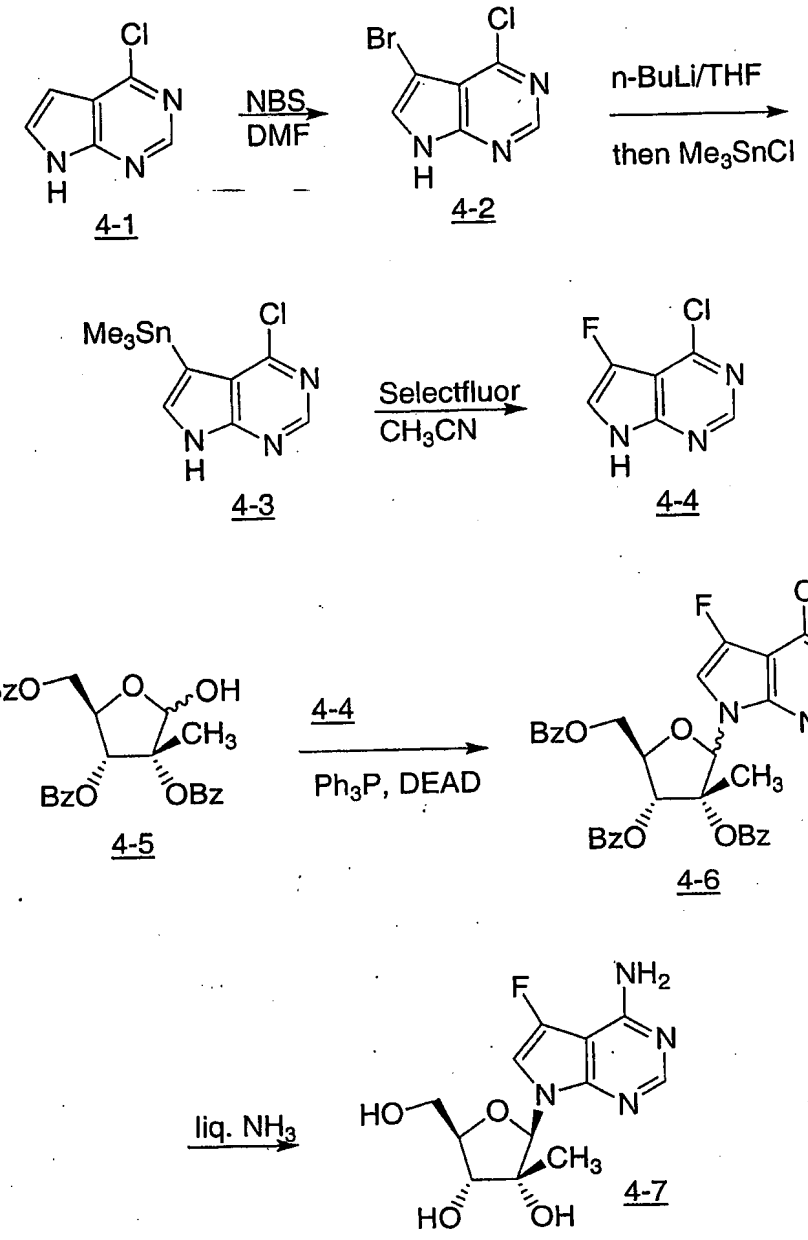
A solution of the compound from Step B (18 mg, 0.026 mmol) and DMAP (3.5 mg, 0.028 mmol) in anhydrous CH_2Cl_2 (300 μL) was cooled in an ice bath for 10 minutes under an argon atmosphere. To this solution was added triethylamine (7.5 μL, 0.053 mmol) followed by octanoyl chloride (6.6 μL, 0.038 mmol). The reaction mixture was stirred at this temperature for 2 h, diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous sodium bicarbonate solution (2 x 10 mL) followed by water (10 mL). The crude product obtained after evaporation was purified by column chromatography on silica gel eluting with 10% acetone in CH_2Cl_2. The title compound was obtained as a colorless foam (13.5 mg).

Step C: 4-Amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3-3)

A solution of the compound from Step B (13 mg, 0.016 mmol) in 6:3:1 MeOH: acetic acid: H_2O (500 μL) was stirred at 50°C for 15 h. The reaction mixture was then concentrated to dryness. The residue was diluted with ethyl acetate (15 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 5 mL) and water (2 x 5 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated. The crude product was purified by silica gel column chromatography eluting with 10% acetone in dichloromethane to afford the title compound as a white foam (6.0 mg).

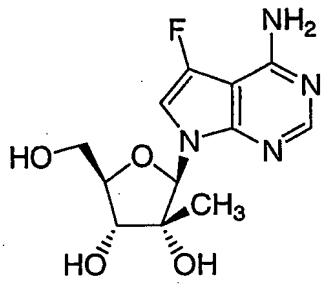
^{1}H NMR ($CDCl_3$): δ 8.29 (s, 1H), 7.25 (d, 1H, J = 3.4 Hz), 6.40 (d, 1H, J = 4.0 Hz), 6.23 (s, 1H), 5.22-5.39 (m, 3H), 4.60-4.39 (m, 4H), 2.47-2.35 (m, 4H), 1.82-1.60 (m, 4H), 1.27 (bs, 16 H), 0.87 (s, 3H), 0.873-0.80 (m, 6H).

Scheme 4



EXAMPLE 40

4-Amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (4-7)



Step A: 5-Bromo-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (4-2)

To a solution of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (4-1) (1.53 g, 10.0 mmol) in DMF (20 mL) was added N-bromosuccinimide (1.78 g, 10.0 mmol) in DMF (10 mL) dropwise at 0°C. The reaction mixture was stirred at 0°C for 30 min and then at room temperature for 1 h. Methanol (25 mL) was added, and the reaction mixture was stirred for an additional 1 h. The solvent was evaporated and the residue was crystallized from methanol to give the title compound as white solid.

Step B: 5-(Trimethylstannyl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (4-3)

To a solution of the compound from Step A (0.92 g, 4 mmol) in THF (25 mL) was added n-BuLi (2.5 M solution in hexane, 3.48 mL) dropwise at –78°C. After the addition, the reaction mixture was stirred at –78°C for an additional 30 min. To this solution was added trimethyltin chloride (0.88 g, 4.4 mmol) in THF (8 mL) dropwise for a period of 10 min. The reaction mixture was brought to room temperature slowly and stirred at room temperature overnight. Saturated aqueous ammonium chloride (60 mL) was added and extracted with ethyl acetate (3 x 70 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified over silica gel to give the title compound as a colorless solid.

Step C: 5-Fluoro-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (4-4)

To a solution of the compound from Step B (1.97 g, 6.20 mmol) in CH_3CN (60 mL) was added [1-(chloromethyl)-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate)] (SELECTFLUOR® fluorinating

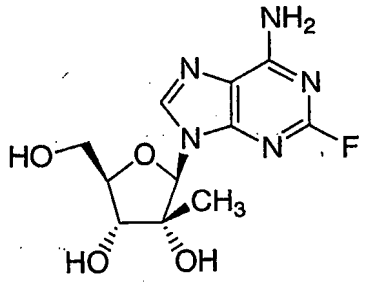
reagent) (2.40 g, 6.5 mmol) in one portion and the reaction mixture was stirred at room temperature for 7 h. The white precipitate was filtered off, and the filtrate was evaporated to dryness. The residue was purified over silica gel using ethyl acetate/hexane (3:7) as the eluent. Fractions containing the product were pooled and eveporated in vacuo to give the title compound as a colorless solid.
^{1}H-NMR (MeOH-d_4): δ 8.53 (s, 1H), 7.37 (d, J = 2.8 Hz);
^{19}F-NMR (DMSO-d_6): δ -171.5.

Step D: 4-Amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (4-7)

To a solution of the compound from Step C (0.075 g, 0.44 mmol), 2,3,5-tri-O-benzoyl-2-*C*-methyl-D-ribofuranose [Harry-O'kuru, Rogers E.; Smith, Jennifer M.; Wolfe, Michael S, "A Short, Flexible Route toward 2'-*C*-Branched Ribonucleosides," J. Org. Chem.,62: 1754-1759 (1997)] (4-5) (0.25 g, 0.53 mmol) and triphenylphosphine (0.23 g, 0.88 mmol) in THF (15 mL) was added diethyl azodicarboxylate (DEAD) (0.14 mL, 0.88 mmol). The reaction mixture was stirred at room temperature overnight. The solution was directly adsorbed onto silica gel and purified over silica gel using ethyl acetate/hexane 1:9 as the eluent Appropriate fractions were dissolved in dioxane (3 mL) and liquid ammonia (4 mL) and the mixture was heated in a steel bomb at 85°C for 24 h. The solvent was evaporated and the residue was purified over silica gel using methanol/dichloromethane (1:9) as the eluent. Fractions containing the desired compound were pooled and evaporated in vacuo to give the title compound.
^{1}H-NMR (MeOH-d_4): δ 8.07 (s, 1H), 7.41 (d, J = 2.2 Hz, 1H), 6.25 (d, J = 1.8 Hz), 4.09-3.95 (m, 3H), 3.82 (dd, J = 2.7, 12.5 Hz, 1H);
^{19}F-NMR (MeOH-d_4): δ -170.4; mass spectrum: 321 $(M+Na)^+$.

EXAMPLE 41

6-Amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine



Step A: 2-Amino-6-chloro-9-(2,3,5-tri-O-benzoyl-2-*C*-methyl-β-D-ribofuranosyl)purine

To a pre-cooled solution of 1,2,3,5-tetra-O-benzoyl-2-*C*-methyl-D-ribofuranose [Harry-O'kuru, Rogers E.; Smith, Jennifer M.; Wolfe, Michael S, "A Short, Flexible Route toward 2'-*C*-Branched Ribonucleosides," J. Org. Chem.,62: 1754-1759 (1997)] (1.74 g, 3.00 mmol) in acetonitrile (15 mL) was added 2-amino-6-chloropurine (0.56 g, 3.30 mmol), then diazabicyclo[5.4.0]undec-7-ene (DBU) (1.37 g, 9.00 mmol), and then dropwise trimethylsilylmethyl trifluoromethanesulfonate (TMS trifate) (2.67 g, 12.00 mmol). The resulting mixture was heated to 65°C for 4 h, then cooled and partitioned between saturated aqueous sodium bicarbonate (200 mL) and dichloromethane (200 mL). The organic phase was dried over magnesium sulfate, filtered and evaporated in vacuo. The resulting crude product was used directly in step B.

Step B: 2-Amino-6-chloro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine

To the crude compound from Step A (2.54 g) in THF (18 mL) was added aqueous 2N LiOH (6 mL). The resulting mixture was stirred at room temperature for 3 h, the THF evaporated in vacuo and the resulting aqueous phase neutralized by addition of aqueous 2N hydrochloric acid. The mixture was adsorbed onto silica gel by evaporation in vacuo and purified on silica gel using

methanol/dichloromethane (1:4) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product as a colorless powder.

Step C: 2,6-Diamino-9-(2-*C*-methyl-β-D-ribofuranosyl)purine

To the compound from Step B (100 mg) was added ammonium hydroxide (5 mL) and the resulting mixture was stirred at 80°C in a Parr bomb overnight. The mixture was cooled and evaporated in vacuo and adsorbed onto silica and purified on silica gel using methanol/dichloromethane (1:4) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product as a colorless powder.

Step D: 6-Amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine

To a mixture of HF/pyridine (70%) (1 mL) in pyridine (1 mL) at -30°C was added the compound from Step C (29.6 mg, 0.10 mmol) in pyridine (0.5 mL), followed by addition of tert-butyl nitrite (0.018 mL, 0.15 mmol). The mixture was stirred for 5 minutes and then poured into ice water (5 mL), neutralized with 2N NaOH and evaporated in vacuo. The crude product was purified on silica gel using methanol/dichloromethane (1:9 through 1:4) as the eluent. Fractions containing the desired product were pooled and evaporated in vacuo to give the desired compound as a colorless oil.

^{1}H-NMR (acetonitrile-d_3): δ 8.23 (s, 1H), 5.93 (s, 1H), (t, J = 8.4 Hz, 1H), 4.00 (m, 2H), 3.81 (m, 1H), 3.70 (s, 1H), 3.60 (m, 1H), 0.90 (s, 3H).

^{19}F-NMR (MeOH-d_4): -80;

Mass spectrum: 298.3 $(M-H)^+$ and 597.1 $(2M-H)^+$.

BIOLOGICAL ASSAYS

The assays employed to measure activity against vaccinia virus are described below:

a. Determination of In Vitro Antiviral Activity of Compounds Against Vaccinia Virus (Cytopathic Effect Inhibition Assay):

Assay conditions are described in the article by Sidwell and Huffman, "Use of disposable microtissue culture plates for antiviral and interferon induction studies," Appl. Microbiol. 22: 797-801 (1971).

Vaccinia virus, Lederle strain from the ATCC, was used with Vero cells and media (9% fetal bovine serum, 0.1% $NaHCO_3$, no antibiotics) as stated in the article. Antiviral test medium was MEM with 2% FBS and 0.18% $NaHCO_3$.

Four and seven point titrations were used to assay compound inhibition. Final compound concentrations for 4-point titrations were 100, 10, 1, and 0.1 μM. Seven-point titrations were performed by preparing one-half log serial dilutions from maximum compound concentrations of either 100 μM or 320 μM. Virus was added to the assay plate approximately 5 min after the test compound. Assay plates were incubated with humidified air and 5% CO_2 at 37°C. Cytotoxicity was monitored in the control cells microscopically for morphologic changes. Regression analysis of the virus CPE data and the toxicity control data gave the ED50 (50% effective dose) and CC50 (50% cytotoxic concentration). The selectivity index (SI) was calculated by the formula: SI = CC50 ÷ ED50.

(S)-1-[3-hydroxy-2-(phosphonylmethoxy)-propyl]cytosine (cidofovir) was used as a positive control for anti-vaccinia virus testing.

Representative compounds tested in the anti-vaccinia virus assay exhibited EC_{50}'s less than 100 micromolar.

b. Determination of In Vitro Antiviral Activity of Compounds Against Vaccinia Virus (Neutral Red Uptake Assay)

After performing the CPE inhibition assay above, an additional cytopathic detection method was used. McManus described the detection method in "Microtiter Assay for Interferon: Microspectrophotometric Quantitation of Cytopathic Effect," Appl. Environ. Microbiol., 31: 35-38 (1976). A Model EL309 microplate reader (Bio-Tek Instruments Inc.) was used at 540 nm to directly read the assay plate. ED50 and CD50 were calculated as above.

The nucleoside derivatives of the present invention were also evaluated for cellular toxicity and antiviral specificity in the counterscreens described below.

COUNTERSCREENS:

The ability of the nucleoside derivatives of the present invention to inhibit human DNA polymerases was measured in the following assays.

a. Inhibition of Human DNA Polymerases alpha and beta:

Reaction Conditions:
50 μL reaction volume

Reaction buffer components:
20 mM Tris-HCl, pH 7.5
200 μg/mL bovine serum albumin
100 mM KCl
2 mM β-mercaptoethanol
10 mM $MgCl_2$
1.6 μM dATP, dGTP, dCTP, dTTP
α-^{33}P-dATP

Enzyme and template:
0.05 mg/mL gapped fish sperm DNA template
0.01 U/μL DNA polymerase α or β

Preparation of gapped fish sperm DNA template:
Add 5 μL 1M $MgCl_2$ to 500 μL activated fish sperm DNA (USB 70076);
Warm to 37°C and add 30 μL of 65 U/μL of exonuclease III (GibcoBRL 18013-011);
Incubate 5 min at 37°C;
Terminate reaction by heating to 65 °C for 10 min;
Load 50-100 μL aliquots onto Bio-spin 6 chromatography columns (Bio-Rad 732-6002) equilibrated with 20 mM Tris-HCl, pH 7.5;
Elute by centrifugation at 1,000Xg for 4 min;
Pool eluate and measure absorbance at 260 nm to determine concentration.

The DNA template was diluted into an appropriate volume of 20 mM Tris-HCl, pH 7.5 and the enzyme was diluted into an appropriate volume of 20 mM Tris-HCl, containing 2 mM β-mercaptoethanol, and 100 mM KCl. Template and enzyme were pipetted into microcentrifuge tubes or a 96 well plate. Blank reactions excluding enzyme and control reactions excluding test compound were also prepared using enzyme dilution buffer and test compound solvent, respectively. The reaction was initiated with reaction buffer with components as listed above. The reaction was incubated for 1 h at 37°C. The reaction was quenched by the addition of 20 μL 0.5M

EDTA. 50 μL of the quenched reaction was spotted onto Whatman DE81 filter disks and air dried. The filter disks were repeatedly washed with 150 mL 0.3M ammonium formate, pH 8 until 1 mL of wash is < 100 cpm. The disks were washed twice with 150 mL absolute ethanol and once with 150 mL anhydrous ether, dried and counted in 5 mL scintillation fluid.

The percentage of inhibition was calculated according to the following equation: % inhibition = [1-(cpm in test reaction - cpm in blank)/(cpm in control reaction - cpm in blank)] x 100.

b. Inhibition of Human DNA Polymerase gamma :

The potential for inhibition of human DNA polymerase gamma was measured in reactions that included 0.5 ng/ μL enzyme; 10 μM dATP, dGTP, dCTP, and TTP; 2 μCi/reaction [α-^{33}P]-dATP, and 0.4 μg/μL activated fish sperm DNA (purchased from US Biochemical) in a buffer containing 20 mM Tris pH8, 2 mM β-mercaptoethanol, 50 mM KCl, 10 mM $MgCl_2$, and 0.1 μg/μL BSA. Reactions were allowed to proceed for 1 h at 37°C and were quenched by addition of 0.5 M EDTA to a final concentration of 142 mM. Product formation was quantified by anion exchange filter binding and scintillation counting. Compounds were tested at up to 50 μM.

The percentage of inhibition was calculated according to the following equation: % inhibition = [1-(cpm in test reaction - cpm in blank)/(cpm in control reaction - cpm in blank)] x 100.

The ability of the purine nucleoside derivatives of the present invention to inhibit HIV infectivity and HIV spread was measured in the following assays.

c. HIV Infectivity Assay

Assays were performed with a variant of HeLa Magi cells expressing both CXCR4 and CCR5 selected for low background β-galactosidase (β-gal) expression. Cells were infected for 48 h, and β-gal production from the integrated HIV-1 LTR promoter was quantified with a chemiluminescent substrate (Galactolight Plus, Tropix, Bedford, MA). Inhibitors were titrated (in duplicate) in twofold serial dilutions starting at 100 μM; percent inhibition at each concentration was calculated in relation to the control infection.

d. Inhibition of HIV Spread

The ability of the compounds of the present invention to inhibit the spread of the human immunedeficiency virus (HIV) was measured by the method described in U.S. Patent No. 5,413,999 (May 9, 1995), and J.P.Vacca, et al., Proc. Natl. Acad. Sci., 91: 4096-4100 (1994), which are incorporated by reference herein in their entirety.

The nucleoside derivatives of the present invention were also screened for cytotoxicity against cultured hepatoma (HuH-7) cells containing a subgenomic HCV Replicon in an MTS cell-based assay as described in the assay below. The HuH-7 cell line is described in H. Nakabayashi, et al., Cancer Res., 42: 3858 (1982).

e. Cytotoxicity assay:

Cells were plated at 15-20,000 cells/well in 100 μL of appropriate media and incubated 18 h at 37°C, 5% CO_2. 100 μL of compound diluted in complete media was added to the cells for a final of 1% DMSO concentration. The plates were incubated at 37°C and 5% CO_2 for 24 h. After the incubation period, 40 μL of CellTiter 96 Aqueous One Solution Cell Proliferation Assay reagent (MTS) (Promega) was added to each well, and the plates were incubated at 37°C and 5% CO_2 for 1 h. The plates were agitated to mix well and absorbance at 490 nm was read using a plate reader. Metabolically active cells reduce MTS to formazan. Formazan absorbs at 490 nm. The absorbance at 490 nm in the presence of compound was compared to absorbance in cells without any compound added.
Reference: Cory, A. H. *et al.*, "Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture," Cancer Commun. 3: 207 (1991).

EXAMPLE OF A PHARMACEUTICAL FORMULATION

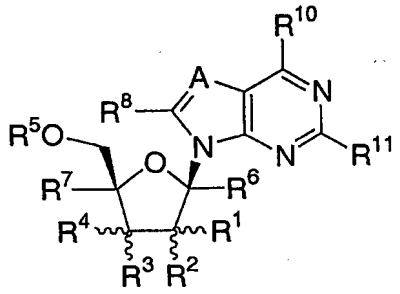
As a specific embodiment of an oral composition of a compound of the present invention, 50 mg of the compound of Example 2 is formulated with sufficient finely divided lactose to provide a total amount of 580 to 590 mg to fill a size O hard gelatin capsule.

While the invention has been described and illustrated in reference to specific embodiments thereof, those skilled in the art will appreciate that various changes, modifications, and substitutions can be made therein without departing from

the spirit and scope of the invention. For example, effective dosages other than the preferred doses as set forth hereinabove may be applicable as a consequence of variations in the responsiveness of the human being treated for severity of the HCV infection. Likewise, the pharmacologic response observed may vary according to and depending upon the particular active compound selected or whether there are present pharmaceutical carriers, as well as the type of formulation and mode of administration employed, and such expected variations or differences in the results are contemplated in accordance with the objects and practices of the present invention. It is intended therefore that the invention be limited only by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.

WHAT IS CLAIMED IS:

1. A method of inhibiting orthopoxvirus replication comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound of structural formula I:



(I)

or a pharmaceutically acceptable salt thereof; wherein

A is N or C-R^9;

R^1 is C_{2-4} alkenyl, C_{2-4} alkynyl, or C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^2 is amino, fluorine, hydroxy, C_{1-10} alkylcarbonyloxy, mercapto, or C_{1-4} alkoxy;

R^3 and R^4 are each independently selected from the group consisting of hydrogen, cyano, azido, halogen, hydroxy, C_{1-16} alkylcarbonyloxy, C_{2-18} alkenylcarbonyloxy, C_{1-10} alkyloxycarbonyloxy, C_{3-6} cycloalkylcarbonyloxy, C_{3-6} cycloalkyloxycarbonyloxy, mercapto, amino, C_{1-4} alkoxy, C_{2-4} alkenyl, C_{2-4} alkynyl, and C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^5 is hydrogen, C_{1-16} alkylcarbonyl, C_{2-18} alkenylcarbonyl, C_{1-10} alkyloxycarbonyl, C_{3-6} cycloalkylcarbonyl, C_{3-6} cycloalkyloxycarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or $P(O)R^{13}R^{14}$;

R^6 and R^7 are each independently hydrogen, methyl, hydroxymethyl, or fluoromethyl;

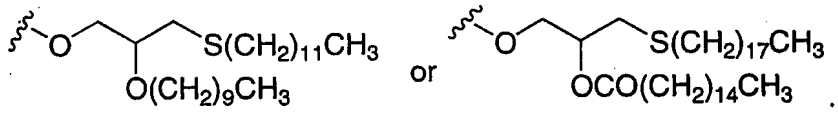
R^8 is hydrogen, C_{1-4} alkyl, C_{2-4} alkynyl, halogen, cyano, carboxy, C_{1-4} alkyloxycarbonyl, azido, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, hydroxy, C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfonyl, or (C_{1-4} alkyl)$_{0-2}$ aminomethyl;

R^9 is hydrogen, cyano, nitro, $NHCONH_2$, $CONR^{12}R^{12}$, $CSNR^{12}R^{12}$, $COOR^{12}$, $C(=NH)NH_2$, hydroxy, C_{1-3} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, halogen, or C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with one to three groups independently selected from halogen, amino, hydroxy, carboxy, and C_{1-3} alkoxy;

R^{10} and R^{11} are each independently hydrogen, hydroxy, mercapto, halogen, C_{1-4} alkoxy, C_{1-4} alkylthio, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{3-6} cycloalkylamino, di(C_{3-6} cycloalkyl)amino, phenyl-C_{1-2} alkylamino, C_{1-4} acylamino, C_{1-8} alkylcarbonyloxy, or $OCH(C_{1-4}$ alkyl$)O(C=O)C_{1-4}$ alkyl;

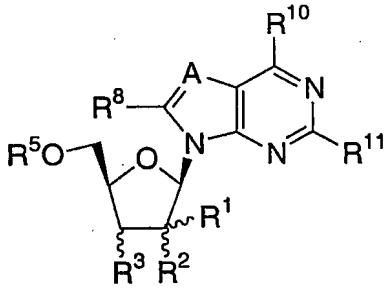
each R^{12} is independently hydrogen or C_{1-6} alkyl; and

R^{13} and R^{14} are each independently hydroxy, $OCH_2CH_2SC(=O)C_{1-4}$ alkyl, $OCH_2O(C=O)OC_{1-4}$ alkyl, $NHCHMeCO_2Me$, $OCH(C_{1-4}$ alkyl$)O(C=O)C_{1-4}$ alkyl,



2. A method of treating orthopoxvirus infection in a mammal in need thereof comprising administering a therapeutically effective amount of a compound of Claim 1.

3. The method of Claim 1 wherein the compound is of structural formula II:



(II)

or a pharmaceutically acceptable salt thereof; wherein

A is N or C-R^9;

R^1 is C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-3} alkoxy, C_{1-3} alkylthio, or one to three fluorine atoms;
R^2 is hydroxy, C_{1-16} alkylcarbonyloxy, fluoro, or C_{1-3} alkoxy;
R^3 is hydrogen, halogen, hydroxy, C_{1-16} alkylcarbonyloxy, amino, or C_{1-3} alkoxy;
R^5 is hydrogen, C_{1-16} alkylcarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or PO_3H_2;
R^8 is hydrogen, amino, or C_{1-4} alkylamino;
R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and
R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino.

4. The method of Claim 3 wherein
R^1 is methyl, fluoromethyl, hydroxymethyl, difluoromethyl, trifluoromethyl, or aminomethyl;
R^2 is hydroxy, C_{1-16} alkylcarbonyloxy, fluoro, or methoxy;
R^3 is hydrogen, fluoro, hydroxy, C_{1-16} alkylcarbonyloxy, amino, or methoxy;
R^5 is hydrogen, C_{1-16} alkylcarbonyl, or $P_3O_9H_4$;
R^8 is hydrogen or amino;
R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and
R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino.

5. The method of Claim 4 wherein the compound is selected from the group consisting of:
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-methylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-dimethylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile,

4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2,4-diamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-hydroxypurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-cyclopropylaminopurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2,6-diaminopurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-methylaminopurine,
6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine,
2'-*C*-methyl-adenosine,
4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine, and
4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

6. The method of Claim 5 wherein the compound is selected from the group consisting of:
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine,
2'-*C*-methyl-adenosine,

4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine, and
4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

7. The method of Claim 6 wherein the compound is 4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine; or a pharmaceutically acceptable salt thereof.

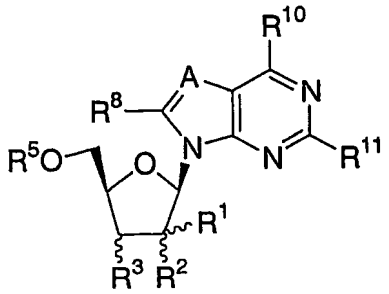
8. The method of Claim 6 wherein the compound is 4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine; or a pharmaceutically acceptable salt thereof.

9. The method of Claim 6 wherein the compound is 4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine; or a pharmaceutically acceptable salt thereof.

10. The method of Claim 6 wherein the compound is 4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine; or a pharmaceutically acceptable salt thereof.

11. The method of Claim 6 wherein the compound is 4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine; or a pharmaceutically acceptable salt thereof.

12. The method of Claim 2 wherein the compound is of structural formula II:



(II)

or a pharmaceutically acceptable salt thereof; wherein

A is N or C-R^9;

R^1 is C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-3} alkoxy, C_{1-3} alkylthio, or one to three fluorine atoms;

R^2 is hydroxy, C_{1-16} alkylcarbonyloxy, fluoro, or C_{1-3} alkoxy;

R^3 is hydrogen, halogen, hydroxy, C_{1-16} alkylcarbonyloxy, amino, or C_{1-3} alkoxy;

R^5 is hydrogen, C_{1-16} alkylcarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or PO_3H_2;

R^8 is hydrogen, amino, or C_{1-4} alkylamino;

R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and

R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino.

13. The method of Claim 12 wherein

R^1 is methyl, fluoromethyl, hydroxymethyl, difluoromethyl, trifluoromethyl, or aminomethyl;

R^2 is hydroxy, C_{1-16} alkylcarbonyloxy, fluoro, or methoxy;

R^3 is hydrogen, fluoro, hydroxy, C_{1-16} alkylcarbonyloxy, amino, or methoxy;

R^5 is hydrogen, C_{1-16} alkylcarbonyl, or $P_3O_9H_4$;

R^8 is hydrogen or amino;

R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and

R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino.

14. The method of Claim 13 wherein the compound is selected from the group consisting of:

4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-methylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-dimethylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile,
4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2,4-diamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-hydroxypurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-cyclopropylaminopurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2,6-diaminopurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-methylaminopurine,
6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine,
2'-*C*-methyl-adenosine,
4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine, and
4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

15. The method of Claim 14 wherein the compound is selected from the group consisting of:

4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine,
2'-*C*-methyl-adenosine,
4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine, and
4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

16. The method of Claim 15 wherein the compound is
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

17. The method of Claim 15 wherein the compound is
4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

18. The method of Claim 15 wherein the compound is
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

19. The method of Claim 15 wherein the compound is
4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

20. The method of Claim 15 wherein the compound is 4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine; or a pharmaceutically acceptable salt thereof.

21. The method of Claim 1 wherein said orthopoxvirus replication is vaccinia virus or variola virus replication.

22. The method of Claim 2 wherein said orthopoxvirus infection is vaccinia virus or variola virus infection.

23. The method of Claim 22 in combination with a therapeutically effective amount of another agent active against orthopoxvirus.

24. The method of Claim 23 wherein said agent active against orthopoxvirus is cidofovir, ribavirin, levovirin, or viramidine.

25. Use of a compound of Claim 1 for the inhibition of orthopoxvirus replication in a mammal.

26. Use of a compound of Claim 1 for the treatment of orthopoxvirus infection in a mammal.

27. The use of Claim 26 wherein said orthopoxvirus infection is vaccinia virus infection or variola virus infection.

28. Use of a compound of Claim 1 in the manufacture of a medicament for the inhibition of orthopoxvirus replication in a mammal.

29. Use of a compound of Claim 1 in the manufacture of a medicament for the treatment of orthopoxvirus infection in a mammal.

30. The use of Claim 29 wherein said orthopoxvirus infection is vaccinia virus or variola virus infection.

31. The method of Claim 8 wherein the orthopoxvirus replication is vaccinia virus or variola virus replication.

32. The method of Claim 17 wherein the orthopoxvirus replication is vaccinia virus or variola virus replication.

33. A compound which is 6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/03703

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/7052, 31/7076, 31/708
US CL : 514/43, 45, 46, 47, 48
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/43, 45, 46, 47, 48; 536/27.1, 27.13, 27.14, 27.2, 27.21, 27.22, 27.23, 27.3, 27.4, 27.6, 27.61, 27.63, 27.7, 27.8, 27.81

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST, STN (CAS ONLINE, MEDLINE, BIOSIS)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 01/90121 A2 (NOVIRIO PHARMACEUTICALS LIMITED) 29 November 2001 (29.11.2001), entire document.	1-33
Y	WO 01/92282 A2 (NOVIRIO PHARMACEUTICALS LIMITED) 06 December 2001 (06.12.2001), entire document.	1-33
Y,P	WO 02/18404 A2 (F. HOFFMANN-LA ROCHE AG) 07 March 2002 (07.03.2002), entire document.	1-33
Y,P	WO 02/32920 A2 (PHARMASSET LIMITED) 25 April 2002 (25.04.2002), entire document.	1-33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"&" document member of the same patent family

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11 July 2003 (11.07.2003)

Date of mailing of the international search report
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